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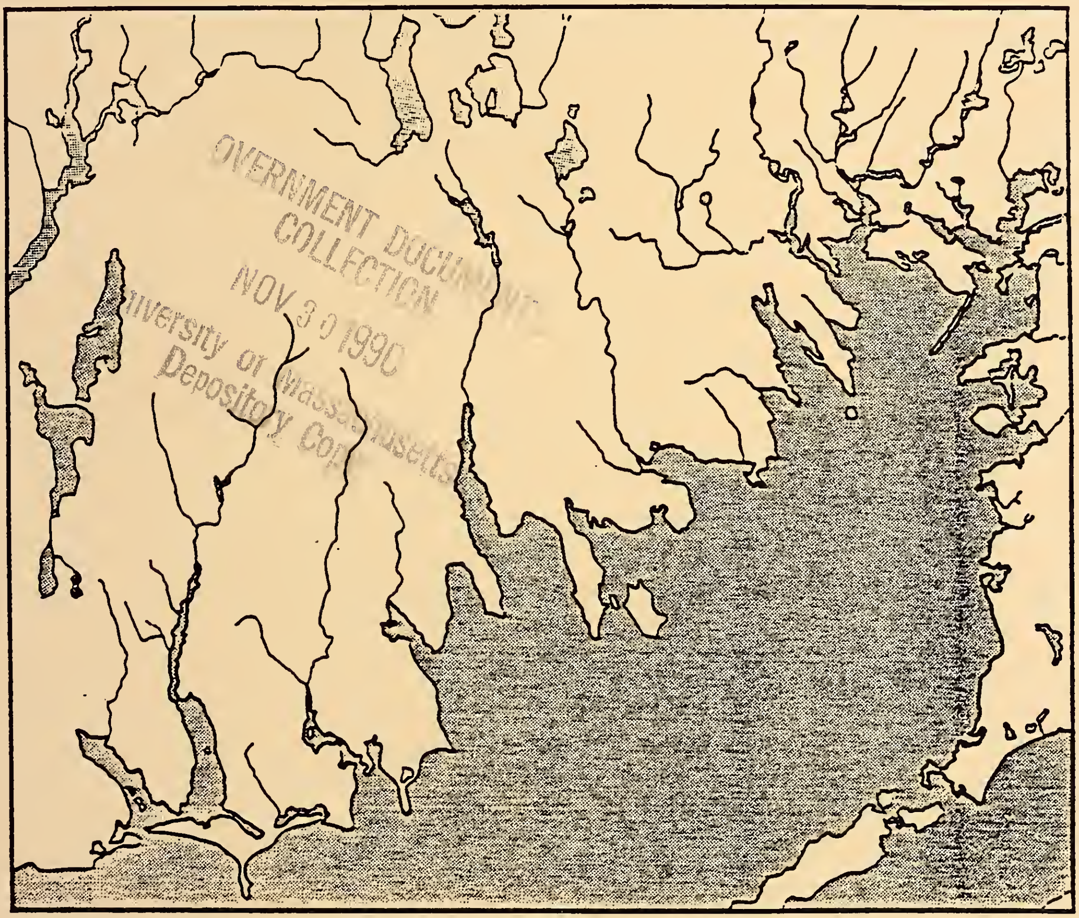

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BUZZARDS BAY RESEARCH

INVESTIGATION OF DIFFERENT BACTERIAL INDICATORS TO EVALUATE NONPOINT SOURCES OF FECAL CONTAMINATION

1986

Prepared by

Joan L. Beskenis
Environmental Analyst III

Technical Services Branch
Division of Water Pollution Control
Westborough, MA

EXECUTIVE OFFICE OF ENVIRONMENTAL AFFAIRS
John P. DeVillars, Secretary

DEPARTMENT OF ENVIRONMENTAL PROTECTION
Daniel S. Greenbaum, Commissioner

DIVISION OF WATER POLLUTION CONTROL
Arleen O'Donnell, Acting Director

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DATE: March 1990

AUTHOR: Joan L. Beskenis

REVIEWED BY:

Arthur S. Johnson

Arthur S. Johnson
Biomonitoring Program
Manager

APPROVED BY:

Alan N. Cooperman

Alan N. Cooperman
Supervising Environmental
Engineer

FOREWORD

The Massachusetts Division of Water Pollution Control was established by the Massachusetts Clean Water Act, Chapter 21 of the General Laws as amended by Chapter 685 of the Acts of 1966. Included in the duties and responsibilities of the Division is the periodic examination of the water quality of various coastal waters, rivers, streams, ponds, and wetlands of the Commonwealth, as stated in section 27, paragraph 5 of the Acts. This section further directs the Division to publish the results of such examinations together with the standard of water quality established for the various waters. One of the responsibilities of the Technical Services Branch of the Division of Water Pollution Control is the execution of this directive. This report is published under the Authority of the Acts and is among a continuing series of reports issued by the Division presenting water quality data and analyses, water quality management plans, baseline and intensive limnological studies and various special studies.

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Technical Services Branch
Westview Building, Lyman School
Westborough, MA 01581

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1.0 EXECUTIVE SUMMARY

This study was initiated in 1986 and was partially funded by the U.S. Environmental Protection Agency. During the planning stages for this study EPA had only recently issued their microbiological criteria for bathing beaches (US EPA, 1986). This resulted from epidemiological studies done by Cabelli (1983) and Dufour (1984). Risk levels were determined by them for "highly credible gastrointestinal illness" using either enterococci bacteria or E. coli bacteria.

When this study began, individuals from state and federal agencies were encouraged and indeed trained in the use of the enterococci and E. coli tests at EPA sponsored workshops and via their handout Test Methods for Escherichia coli and Enterococci in Water by the Membrane Filter Procedure (USEPA, 1985). These indicators can be contributed by all kinds of warmblooded animals including cows, dogs, humans but the health effects studies were conducted at areas with contributions primarily from human sewage. Risk levels from nonpoint sources were not determined. Nonpoint sources can contain human waste products, but it may also be dominated by the waste of other animals.

Nonpoint sources were examined at areas in Buzzards Bay for both their impact on water quality and on closure of shellfish areas. Some of the nonpoint sources had the potential for transporting human waste (marinas, septic systems) while others (marshes, birds, agricultural land) were essentially non-human sources. The risk levels for intestinal diseases from non-human fecal contributors is not well known. Unfortunately, although an epidemiological study was planned by EPA to be conducted simultaneously with the microbial indicator surveys at shellfish areas, one was not done at that time. Instead, methods for evaluating the data on microbial inputs from nonpoint source areas were at the discretion of the principal investigator. The principal investigator could evaluate relative contributions of significant fecal indicator organisms from nonpoint sources. But, until an epidemiological study is done at areas dominated by nonpoint sources, then the level of risk cannot be known.

The approach for the Buzzards Bay study design was to sample various indicator organisms using "new" media (i.e., mE - enterococci, mTEC - E. coli, mCP - Clostridium perfringens), plus the typical mFC for fecal coliform bacteria and A-1 for fecal coliform bacteria MPN test. From these data a baseline could be established for use in defining problem areas as other bacterial criteria were issued. Also , personnel at the state laboratory (Lawrence Experiment Station) could obtain some experience in running these tests which would be useful in evaluating the feasibility of routinely conducting them in the future.

Still no benchmarks were available with which to evaluate these data. To address this problem it was decided to include source differentiation of the fecal streptococci bacteria to attempt to learn what type of source was impacting an area. For example, if runoff from an upstream farm had a high density of Streptococcus bovis from warmblooded agricultural animals was S. bovis still isolated in a shellfish area downstream? Although causality can not be established in this manner a link between source and fecal contamination can be made. In addition to source differentiation, samples were collected at stations adjacent to and downstream from different types of sources using the various indicator organisms. This was done to determine the relative contributions from each type of source. The ultimate goal is to target remedial actions based on the sources identified. This was the first time that source differentiation of the fecal streptococci was done by the Department of Environmental Protection, and while some success was noted, it was clear that modifications are necessary. The study design has been modified where possible to address these shortcomings.

Some problems which had to be dealt with were: lack of bacterial input from some nonpoint sources during the study period, lack of isolation of the fecal streptococci bacteria, high false positives with the KF medium and incomplete information from stations because only a portion of the fecal streptococci counts (i.e., 5 colonies per station) were analyzed. Lack of distinct streptococci assemblages from the various nonpoint sources examined plus the lack of recovery of S. bovis and S. equinus limit the usefulness of source differentiation. Also, the unpredictability of bacterial counts from nonpoint sources created a problem since so much depends on physical processes such as slopes, soil types, vegetation and rainfall. Sample collection posed problems as well. Although it can be predicted that an area is a good candidate to be impacted by different species of fecal streptococci bacteria because of its proximity to various types of sources, it is difficult to know where, when, and how to sample. In some areas the sampling regime was adequate, while in others improvements needed to be made. The sampling was done during June to October 1986.

Agricultural Areas

In the agricultural area along the Westport River, S. bovis and S. equinus, which are likely to be present in the feces of livestock, could not be isolated during the 1986 sampling. However, animal excrement was much in evidence. The tributaries, particularly Snell Creek, had elevated bacterial counts which degraded the water quality of the river. (Because of these elevated counts, the area was sampled again during 1987). It was found that the counts were elevated at the headwaters of Snell Creek where cows were observed walking through the creek. In 1986, a survey was done during wet weather (October 2, 1986) which demonstrated very high fecal coliform levels. When source differentiation of the fecal streptococci was done it was found that 90 percent were contributed by warmblooded sources. The need for management of the livestock and their wastes was evident.

Residential Areas

The lower segment of the Westport River below Hix Bridge is much more residential than the area above. In this residential area an effort was made to determine if septic tank leaching fields located within 50 feet of the river would have a measurable effect on the in-stream fecal coliform bacteria. It was found that fecal coliform inputs from the houses could not be detected (at significant levels) in the surface waters. This was following a sampling regime of grab samples collected at least two hours after a high tide. The literature suggests that even in sandy soils bacteria do not travel far in a septic tank leaching field. To conclusively show that no bacterial impacts were caused by travel of bacteria through sandy soil, one improvement for instream sampling would be to sample when the probability is high that toilet facilities, dishwashers, clothes washers, are currently being used. Although an effort was made to look for evidence that people were present (i.e., cars etc.) there was no way of confirming if this was true.

Bird Populations

Many informal complaints were received by the sampling crews from local citizens regarding bird populations on the Wareham River and in Muddy Cove, but flocks of birds (e.g., gulls, geese, ducks) were not observed during the sampling events. The time of sample collection was important. In the early summer when the sampling on the Wareham River was conducted, the birds were most likely nesting on shore. On July 21 only 2 percent of the streptococci were likely contributed by birds. Later, on August 11 when Muddy Cove was sampled, 15.7 percent of the fecal streptococci were contributed by Group Q streptococci whose likely source is birds. In September, 22.9 percent of the fecal streptococci colonies analyzed from the Westport River were Group Q streptococci. Whether this increase in percentage over time is a result of changes in bird habits over the course of the summer or whether these percentages are typical of the relative contributions at each site can not be determined from these data. Sampling during the different seasons at each site would need to be conducted to learn if the fecal contributions from birds increased or decreased with the seasons.

Marsh Areas

The only marsh area where fecal coliform counts during the 1986 sampling were elevated was Muddy Cove, Wareham. Dye studies need to be done here to establish circulation patterns and time-of-travel. There was evidence of fecal material from boats in one tidal channel. The source of this material could not be determined (i.e., whether from boats discharging in the Cape Cod Canal - including the large ferry boats, or whether a boater or boaters in the cove discharged their "heads" in the vicinity).

At Muddy Cove the sampling stations closest to shore exhibited the highest bacterial counts. The bacterial counts were already elevated at Gibbs Brook, the freshwater inlet to Muddy Cove. At Station 1212, which was located on Gibbs Brook, just north of Cranberry Highway, the fecal coliform count on August 8 was 200/100 ml. Counts were even higher at the near-shore stations in the unnamed brook located on the western shore. Stations 1201, 1202, 1203 had 335, 365, 400 FC CFU/100 ml respectively. Again, circulation information may help to separate nonpoint sources located along the shore from those more remotely located.

It was concluded from the enterococci/streptococci correlations and plots for this area that meaningful information was not being obtained by this technique. Source differentiation results showed that a variety of sources contributed to this area including warmblooded animals, birds, and insects. Thus, since not all the streptococci were enterococci a strong, significant correlation was not expected. The results from samples collected at this area did not present a clear picture as to how the correlations could be used.

Marinas

Despite limited survey work that was done at marinas, there is evidence of bacterial problems occurring in and around them. This situation might be improved by the construction and use of pump-out facilities at marinas along the Massachusetts coast. As can be noted from the sampling at Warrs Marina in

Wareham, just having a pump-out facility available does not insure its use. Here, a boater, who lived on his boat, was found to be discharging directly into the Wareham River even though a pump-out facility was offered at the marina.

At the other marinas where sampling was done (i.e., Point Independence Yacht Club and Onset Bay Marina) higher fecal coliform counts were found within the slipways as compared to the bay water. In a report issued by S.E. Regional Planning Commission in 1981 entitled: Marine Waste Pump-out Feasibility Study it was projected that 2,500 gallons of waste per week from boats could be pumped from boats which they recommended if the facilities were constructed. Given the lack of facilities at the present time, here and throughout the Buzzards Bay coast, marinas with vessels large enough to have installed marine heads remain an important source of fecal contamination to coastal areas.

Laboratory Study

It was expected that, because of the resuscitation time, E. coli recovered using the mTEC medium and procedure would have higher recoveries than the fecal coliform bacteria recovered with the mFC medium and procedure. Out of a total of 264 instances the E. coli were higher than the fecal coliform only 93 times while the fecal coliform were higher 118 times. The rest of the time their results were equal.

When a graphical comparison of MPN/membrane filter results was done on probability paper on samples from saline waters slightly higher estimates of fecal contamination were found from the membrane filter procedures as opposed to the A-1 MPN procedures. This funding requires further study.

Conclusions

The microbiological sampling of Buzzards Bay incorporated some experimental approaches to the evaluation of nonpoint sources of fecal contamination. In some areas such as Wareham River and Muddy Cove, problems were documented but sources could not be identified. The major sources of bacteria appear to be (1) boats in marina areas (2) overland runoff from livestock and agricultural areas. At the marinas and agricultural areas some success was obtained in identifying sources via the elevated densities of the bacterial indicators. Source differentiation, however, did not provide information that would allow an investigator to separate one type of source from another. In residential areas nutrients rather than bacteria may be a problem. Further refinement of the techniques and methods used during this first microbiological study to examine nonpoint sources of fecal contamination is needed.

These refinements should include: increasing the number of fecal streptococci colonies that are biochemically differentiated; including sampling during other seasons; using a different medium for fecal streptococci to try to reduce the number of false positives.

2.0 INTRODUCTION

The ever-increasing acreage of shellfish beds closed because of fecal contamination from nonpoint sources and point sources alike has intensified the need to locate the sources of this contamination. In order to classify shellfish harvesting areas as open or closed for shellfish harvesting, the Massachusetts Division of Marine Fisheries uses the U.S. Food and Drug Administrations (FDA) bacterial criteria (see Table 1). At the time this study was conducted the Division of Water Pollution Control (DWPC) had authority over the shellfish harvesting areas, but DWPC also used the FDA criteria. Shellfish areas are closed for shellfish harvesting if the fecal coliform most probable number (MPN) is greater than 14/100 ml (see Table 1). In 1986, approximately 1547 acres in Buzzards Bay were closed to shellfishing because of nonpoint sources and 8046 acres closed because of point sources (Hickey and Germano, 1986). This study, funded in part by the U.S. Environmental Protection Agency - Buzzards Bay Program, was an attempt to determine through microbiological examination of fecal indicators the types of sources that are impacting shellfish areas at the Wareham River and coastal waters in the town of Wareham and the Westport River, Westport (Figure 1).

Areas chosen for study exhibited mixed land use patterns and different combinations of potential point and nonpoint sources of fecal contamination. The types of areas studied were: marinas, agricultural land, the Wareham Publicly Owned Treatment Works (POTW), marshland, and housing areas. Samples were collected adjacent to, downstream and offshore from these locations under worst-case conditions (see Study-Design, Appendix, Table A). There were two basic elements of the bacterial sampling: 1) use of several microbial indicators of fecal contamination at all locations; 2) use of biochemical testing to do "source differentiation" of the fecal streptococci bacteria at a more limited number of locations. Maps and lists of the sampling locations are included in the Appendix (see Tables A8 to A25, Figures A1 to A21).

The indicators chosen for use in this study were:

mFC - fecal coliform test and membrane filter procedure

mCP - Clostridium perfringens test and membrane filter procedure

mE - enterococci test and membrane filter procedure

mKF - streptococci test and membrane filter procedure

mTEC - Escherichia coli test and membrane filter procedure

A-1 - fecal coliform test and Most Probable Number procedure (see Appendix for methods and procedures used in the analysis).

Results of the different indicator tests are presented in each section.

These indicator organisms can be contributed by many different types of sources including humans and other warmblooded animals such as birds, livestock and small mammals. But "secondary" sources such as vegetation and insects can also contribute to these counts. All of the indicator organisms chosen for study can and do indicate possible warmblooded sources, but source differentiation of

TABLE 1

COLIFORM STANDARDS FOR OVERLYING* WATERS OF SHELLFISH AREAS

OLD

Total Coliform (MPN/100 ml)
0-70 Open (Approved Areas)
70-700 Restricted (Depuration Required)
>700 Closed

NEW

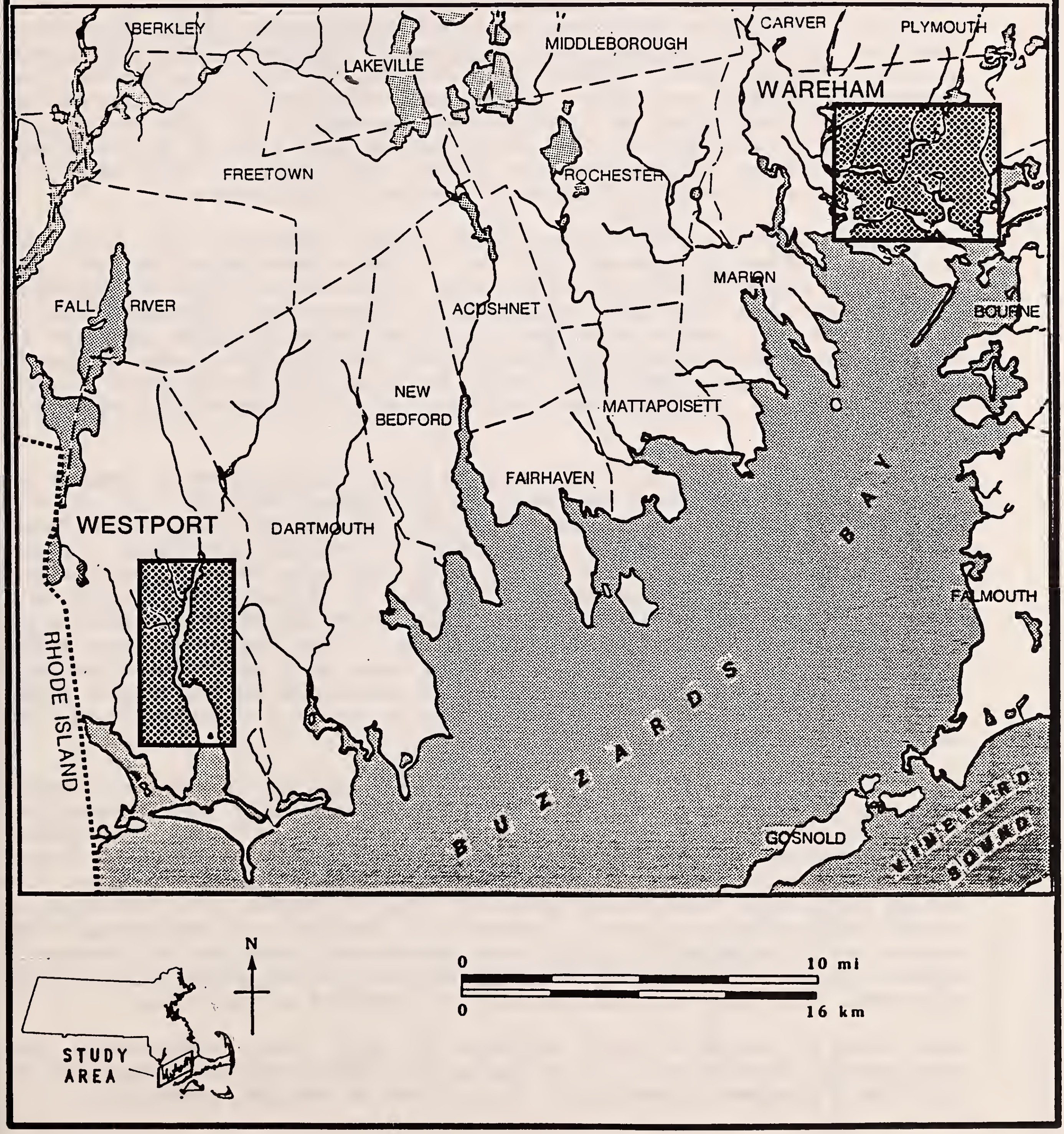
Fecal Coliform (MPN/100 ml)
0-14 Open
14-88 Restricted
>88 Prohibited

---

* Adapted from: National Shellfish Sanitation Program's Manual of Operations. 1988. U.S. Department of Health. U.S. Food and Drug Administration.

Figure 1 SAMPLING PROGRAM AREAS

BUZZARDS BAY, 1986



the fecal streptococci provides one additional level of information which can be important in sorting out community conflicts and lending support to management plans.

The second method selected for evaluating nonpoint sources of fecally derived contamination was to attempt to make use of source differentiation to separate human sources from other warmblooded animal sources, and warmblooded sources from "secondary sources" such as vegetation and insects. So, a series of biochemical tests on the fecal streptococci colonies recovered on the KF media were done. The results from these tests were interpreted according to a method described in EPA's Microbiological Methods for Monitoring the Environment (1978). Through this scheme, contributions of fecal streptococci from warmblooded animals, and birds, insects, and vegetation could be determined (Figure 2). This method of distinguishing sources by the types of group D streptococci present has not been widely used to date although much has been written describing its possible use (Mundt, 1963, Martin, 1972, Rutkowski, 1986). Section 3 contains background information on this method.

In addition to these general descriptions, simple regressions were also done using the statistical package SPSSX and the Lotus spreadsheet on data from areas where source differentiation was done. This was an attempt to determine if there were any significant relationships between fecal streptococci/fecal coliform and also between fecal streptococci/enterococci; and to determine if the predictor line varied significantly from place to place when different types of sources were identified. Graphs of these correlations appear in each section, Table A5 presents correlations from days when source differentiation was done.

Warmblooded animals differ in the amount of fecal coliform bacteria compared to fecal streptococci bacteria in their enteric systems (Table 2). So, if for example, through source differentiation it was learned that most of the fecal streptococci in an area were S. avium, which is contributed by birds, a steep graph might be expected when the fecal streptococci to fecal coliform counts were plotted. Also, the enterococci bacteria (S. faecalis, S. faecium) are isolated by mE media while the KF media isolates the enterococci as well as S. avium and other fecal streptococci species. So, in the case of the birds their excrement would contain both enterococci and other fecal streptococci species. Thus, when the enterococci to streptococci counts are plotted a flat graph might be expected because the enterococci would not be contributed in as high densities as the fecal streptococci.

The predicted graphs might occur if the fecal material was recently contributed and samples were collected near the source, but under typical sampling conditions die-off and dilution of the bacterial indicators occurs. The graphs and correlations appear to be sufficiently altered from the ideal to reduce their use as an indicator of the dominant fecal source impacting an area. If unique graphs were created when different types of sources were identified by source differentiation once a sufficient database was established then biochemical differentiation of the fecal streptococci would not be necessary. However, problems did occur in the interpretation of these graphs which will be discussed. This method of interpretation was abandoned as impractical.

When enteric bacteria (fecal coliform, E. coli, fecal streptococci, and Clostridium) are discharged out of the enteric system they are acted upon by their new environmental conditions. Factors such as sunlight, predation,

FIGURE 2 SCHEMATIC OUTLINE FOR IDENTIFICATION OF FECAL STREPTOCOCCI

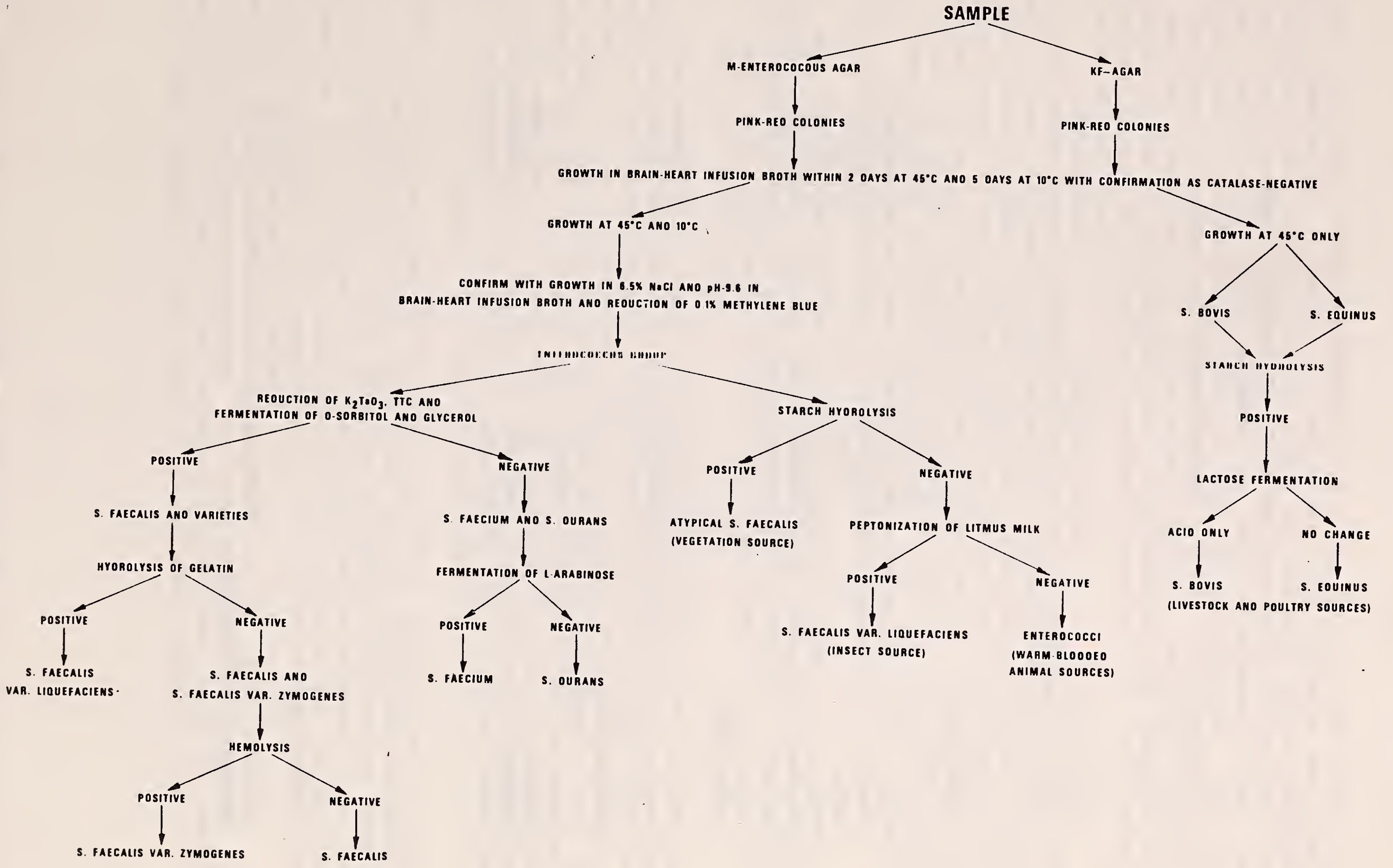

SAMPLE
M-ENTEROCOCOUS AGAR
KF—AGAR
PINK-RED COLONIES
PINK-RED COLONIES
GROWTH IN BRAIN-HEART INFUSION BROTH WITHIN 2 DAYS AT 45°C AND 5 DAYS AT 10°C WITH CONFIRMATION AS CATALASE-NEGATIVE
GROWTH AT 45°C AND 10°C
GROWTH AT 45°C ONLY
CONFIRM WITH GROWTH IN 6.5% NaCl AND pH-9.6 IN BRAIN-HEART INFUSION BROTH AND REDUCTION OF 0.1% METHYLENE BLUE
S. BOVIS
S. EQUINUS
ENTEROCOCCUS GROUP
STARCH HYDROLYSIS
REDUCTION OF K_2TeO_3, TTC AND FERMENTATION OF D-SORBITOL AND GLYCEROL
STARCH HYDROLYSIS
POSITIVE
POSITIVE
NEGATIVE
POSITIVE
NEGATIVE
LACTOSE FERMENTATION
S. FAECALIS AND VARIETIES
S. FAECIUM AND S. DURANS
ATYPICAL S. FAECALIS (VEGETATION SOURCE)
PEPTONIZATION OF LITMUS MILK
ACID ONLY
NO CHANGE
HYDROLYSIS OF GELATIN
FERMENTATION OF L-ARABINOSE
POSITIVE
NEGATIVE
S. BOVIS
S. EQUINUS
(LIVESTOCK AND POULTRY SOURCES)
POSITIVE
NEGATIVE
POSITIVE
NEGATIVE
S. FAECALIS VAR. LIQUEFACIENS (INSECT SOURCE)
ENTEROCOCCI (WARM-BLOODED ANIMAL SOURCES)
S. FAECALIS VAR. LIQUEFACIENS
S. FAECALIS AND S. FAECALIS VAR. ZYMOGENES
S. FAECIUM
S. DURANS
HEMOLYSIS
POSITIVE
NEGATIVE
S. FAECALIS VAR. ZYMOGENES
S. FAECALIS

TABLE 2

ESTIMATES OF MICROBIAL FLORA OF ANIMAL FECES

AVERAGE DENSITY (per gram)

Animal Group	Fecal Coliforms	Streptococci
Farm Animals		
Cow	230,000	1,300,000
Pig	3,300,000	84,000,000
Sheep	16,000,000	38,000,000
Horse	12,600	6,300,000
Duck	33,000,000	54,000,000
Chicken	1,300,000	3,400,000
Turkey	290,000	2,800,000
Animal Pets		
Cat	7,900,000	27,000,000
Dog	23,000,000	980,000,000
Wild Animals		
Mice	330,000	7,700,000
Rabbits	20	47,000
Chipmunk	148,000	6,000,000
Human	13,000,000	3,000,000

Adapted from:

Geldreich, E.E. 1977. Indicators of Virus in Water and Food. Ann Arbor Science, Ann Arbor, MI.

settling of particles, bactericidal effect of algae, all work to reduce the bacterial population. Changes in temperature, pH, nutrients and oxygen availability are also critical to the growth and survival of the indicator organisms. Each group of bacteria have specific requirements for survival with some doing better than others. Within the fecal streptococci group the enterococci tend to survive better than the fecal coliform bacteria. But, also within the fecal streptococci the group from livestock: S. equinus and S. bovis, are very short-lived out of the enteric system. The ideal conditions for survival of the E. coli bacteria are: high nutrients, pH range of 5-9. Also, they are mesophiles so they can reproduce between 10 to 45°C with optimum temperatures between 30-45°C.

In this study, environmental parameters that can affect bacterial survival were measured. These included: pH, temperature, dissolved oxygen, chloride, specific conductance, total hardness, suspended solids, total Kjeldahl-nitrogen, nitrate-nitrogen, ammonia-nitrogen and total phosphorus. However, since there is no way of knowing how long bacteria have been in the water before the sample collection no conclusions can be drawn about their original densities nor about the effect of any parameter on the bacterial viability. Multiple regressions, however, did provide information on where fresh water sources were important and where tidal influences had an impact.

Another aspect of the Buzzards Bay microbial study was to learn from personnel at Lawrence Experiment Station their observations concerning the several microbial indicator organisms included in this study. Personnel from Rosario Grasso's Bacteriology Laboratory reported on colony formation and background growth, among other observations.

Counts of the bacterial indicators E. coli and fecal coliform were compared and graphically represented (Figures 11 to 14) to determine which indicator had the best recovery (or highest counts) in the waters sampled. E. coli (mTEC) is recommended by EPA as the bacterial indicator to use in evaluating freshwater bathing areas, while fecal coliform bacteria (mFC) is currently used in Massachusetts and is the indicator used in the previous EPA bathing standard. The E. coli mTEC test has a resuscitation period of two (2) hours when the incubator temperature is 35°C before it is raised to 44.5°C and it is believed in some circumstances to have better recoveries than that of the fecal coliform membrane filter test (Dufour, Strictland, Cabelli, 1981).

The results of the membrane filter tests for fecal coliform and E. coli bacteria were compared to those of the A-1 MPN test. In marine waters Stevens, Grasso and Delaney, (1977) found that the EC medium MPN test had better recoveries than the FC membrane filter medium and test procedures. Since the constituents in the A-1 medium differ from the EC medium as well as the time needed for confirmation (24 compared to 2 to 4 days respectively), the recoveries of the two media were compared.

The bacterial indicator-enterococci was also sampled; this indicator is proposed by EPA to be used in the evaluation of both fresh water and marine bathing beaches. Comments on this indicator are included in Section 9.0.

3.0 HISTORY AND BACKGROUND INFORMATION ON SOURCE DIFFERENTIATION

During the 1960's and early 1970's, a significant amount of the microbiological work on the environment focussed on culturing and speciating fecal streptococci bacteria. Scientists like Pavlova et al (1971, 1972) and Papavassiliou (1961) worked on improving the media to enhance recovery and the biochemical tests to enhance isolation; Hartman (1966), Deibel (1964) refined the taxonomy, while others tried to apply their work to the interpretation of environmental samples. Bartley and Slanetz (1960) isolated fecal streptococci from feces, sewage and water and presented data on typical biochemical characteristics of the various species. Bayne et al (1983) speciated group D streptococci from above, at, and below a sewer outfall and tried to relate the species to the likely sources. Mundt (1963) examined the occurrence of enterococci on plants and concluded that they were from wild animals and not plant-specific species. Also, Ator and Starzyk (1976) attempted to determine sources of bacteria contributed to the Rock River, Illinois by speciating the fecal streptococci and then grouping the species into likely sources.

The US EPA continued the research on the biochemical tests for species and source differentiation and published improvements upon them (Pavlova et al., 1971) although they have conducted few if any environmental studies using source differentiation. In 1978, the U.S. Environmental Protection Agency published Microbiological Methods for Monitoring the Environment. This contained the schematic for source differentiation and species differentiation which was used (Figure 2) in this study. Table 3 lists species of streptococci bacteria which are included in Group D, the fecal streptococci bacteria.

The Technical Services Branch of the Division of Water Pollution Control used this method somewhat experimentally to identify the usefulness and strengths of source differentiation. It has potential utility to aid in distinquishing the type of bacterial source impacting a particular area.

The types of sources that can be differentiated are:

warmblooded animals
livestock and poultry
insects
vegetation
birds

In the examination of nonpoint sources of fecal contamination these sources may be contributed in differing amounts and pose different levels of risk if they contaminate bathing or shellfish areas.

Currently, shellfishing areas in southeastern Massachusetts are closed for shellfishing when the fecal coliform median exceeds either 14 fecal coliform MPN/100 ml or 70 total coliform MPN/100 ml. The standard for fresh water bathing areas (Class B waters) states that fecal coliform bacteria shall not exceed a log mean of 200/100 ml. For marine bathing beaches the water quality standard is that total coliform bacteria should not exceed a median of 700 MPN/100 ml (see Table 4), however this standard is currently under review and will probably be the same as that for Class B waters in the new revisions.

TABLE 3

SPECIES WITHIN THE FECAL STREPTOCOCCI[*]

GROUP	Species
Enterococci	*S. faecalis*
	S. faecium
	Group Q
Viridans	*S. bovis*
	S. equinus
	S. mitis
	S. salivarius

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[*] Based on Figure from Clausen et al., 1977.

[**] Included in the Group D streptococci

TABLE 4

Massachusetts Microbiological Surface Water Quality Standards for Primary Contact Fresh and Marine Recreational Waters.*

Fresh Water

Class B - Fecal Coliform bacterial shall not exceed a log mean for a set of samples of 200 per 100 ml, nor shall more than 10% of the total samples exceed 400 per 100 ml during any monthly sampling period, except as provided in 314 CMR 4.02(1).

Coastal and Marine Waters

Class SA - Total Coliform bacteria shall not exceed a median value of 70 MPN per 100 ml and not more than 10% of the samples shall exceed 230 MPN per 100 ml in any monthly sampling period.

Class SB - Total Coliform bacteria shall not exceed a median value of 700 MPN per 100 ml and not more than 20% of the samples shall exceed 1000 MPN per 100 ml during any monthly sampling period, except as provided in 314 CMR 4.02(1).

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* Taken from: Massachusetts Surface Water Quality Standards - 1985 Massachusetts Department of Environmental Quality Engineering. Division of Water Pollution Control, Technical Services Branch, Westborough, MA.

It does not matter if the source of the elevated bacterial counts is identified to be human or not. All that is considered is that a given fecal coliform count could represent a certain risk of a person contracting a water-borne or food-borne illness. Like the fecal coliform bacteria the fecal streptococci bacteria are indicator organisms of likely fecal contamination and are not the pathogens. The fecal streptococci may be contributed by point or nonpoint sources of pollution.

Public Health Risks from Point and Nonpoint Sources

In the 1970's, the U.S. Environmental Protection Agency (1986) sponsored epidemiological studies to determine risk levels of contracting "highly credible gastrointestinal illness" - (HCGI) at marine and freshwater bathing beaches. The areas included for study focussed on beaches receiving point sources of contamination. These included marine beaches at New York City, Boston Harbor, MA , and freshwater beaches at Lake Erie, PA and Keystone Lake in Oklahoma. Risk levels of contracting HCGI were determined with various indicator organisms such as enterococci bacteria, *E. coli*, fecal coliform, and *Clostridium perfringens* (Cabelli, 1983). Point sources of fecal contamination were also the primary source of fecal contamination when Kehr et al (1941) did their epidemiological study on shellfish areas at Raritan Bay, New Jersey. Their study is the basis of the current microbiological shellfish standard. The risk levels of contracting human illness from swimming in waters or eating shellfish from areas contaminated by nonpoint sources of fecal contamination, have not been determined. An epidemiological study on shellfish areas is currently underway and is being conducted by the U.S. EPA.

Risk levels of contracting human gastrointestinal illness from nonpoint sources of fecal contamination have not yet been quantified, but it is likely that they will be lower than point sources and will vary based upon the type of source. Most point sources discharge human waste while nonpoint sources may be from humans, other warmblooded animals (including livestock and birds), insects or vegetation. These are the types of sources distinguished by using source differentiation of the fecal streptococci.

Fecal material from warmblooded animals, especially humans, represents the highest risk to humans if it enters water bodies or shellfish beds. There are many bacterial pathogens and viral particles which may be present in human feces, these can cause water-borne and food-borne illness if ingested by humans. The bacterial pathogens *Salmonella* sp. and *Vibrio cholerae*, for example, can cause gastrointestinal upset and cholera respectively. While, infectious hepatitis and gastroenteritis can be caused by hepatitis A virus and Echo viruses, respectively.

Risks to humans from feces from dogs, cats and other small mammals are not as well defined, but still likely includes some bacterial diseases. Viruses however, are not typically transferred interspecies. Many nonpoint sources such as parking lots, storm drains and certain types of marshland may be dominated by non-human but warmblooded sources of fecal streptococci.

During planning meetings for this Buzzard's Bay study the Wareham River and Muddy Cove, Wareham were mentioned as areas where water quality may have been impacted by large populations of birds. Some measure of the bacterial inputs from birds can be determined by the analysis for Streptococcus avium. This fecal streptococci species has many, but not all, of the characteristics of the Group-D fecal streptococci and is placed, at present, into tentative Group Q. Birds, like other warmblooded animals, pose some degree of risk if their excrement enters water bodies or food sources. Recently, non-01 type Vibrio cholerae which can cause "cholera-like" illness in humans was isolated in feces collected from aquatic birds in Colorado and Utah (Ogg et al, 1989).

Group D streptococci are commonly obtained from various parts of insects. Geldreich et al (1964) found them in high numbers from members of the orders Diptera and Coleoptera, both orders are represented in fresh and salt water marshes. The appearance of fecal streptococci on plants coincides with periods of increased movement of insects. Thus, insects may be influential in distributing fecal streptococci away from where it is first deposited. The public health significance of fecal streptococci species associated with insects is not known.

Vegetation can also be a source of fecal streptococci. Again, as with insects, the public health significance is not known and probably differs with the species isolated and their source. Mundt (1963) when studying plants from remote areas of the Great Smokies, away from areas of potential human impact, found enterococci on 14% of almost 2200 flowers sampled. It was concluded from this study that the enterococci were contributed by wild animals and do not represent plant specific species or variants of enterococci. When Mundt (1961) examined agricultural plants he reported that 58.8% of 106 samples were positive for enterococci.

Some fecal streptococci may be contributed by plants which have unique biotype characteristics. Geldreich et al (1964) detected starch hydroloysis in 37.7% of 646 streptococci isolated from plants. This characteristic does not appear to be common to strains recovered from warmblooded animals or insects. The starch hydrolysis test is used to distinguish vegetative sources according to the U.S. EPA (1978) (see Figure 2). There is likely no sanitary significance of these fecal streptococci, although their presence on food stuffs of high starch content may limit the use of a fecal streptococci test for processed vegetables (Clausen, 1977).

Thus each sample with fecal streptococci present provides information for further interpretation. If, for example, an area is contaminated by fecal streptococci contributed by birds rather than by warmblooded animals, the methods used to reduce the bacterial input would differ.

4.0 MARSH, COVE AND BAY SITES

"Wetland areas" were one of the land use types that was examined for possible unique contributions of indicator organisms. Specifically, salt water marshes were chosen for study. Wetlands have been shown to function as sinks or exporters of nutrients during certain times of the year (Bowden, 1982). It has been questioned however, whether the wetland systems function in a similar manner with bacteria. Of special interest is whether indicator organisms of fecal contamination might be "exported" by the marsh.

It was expected that a unique assemblage of fecal streptococci organisms might be isolated at the salt marsh because of the special functions performed by wetlands. S. avium or group Q streptococci was expected because of the nesting, feeding and general life activities of birds in these areas. Streptococci from insects and vegetation also seemed likely to be present in higher amounts than in other areas. Contributions from warmblooded animals were also expected, but perhaps in lower amounts than from agricultural areas.

The areas included in this investigation were Muddy Cove (Aug. 11) Sunset Cove (Aug. 26 and Sept. 24) and Broad Marsh Cove (Oct. 8), all in Wareham. These sites were chosen because they exhibited at least seasonally elevated bacterial counts (Department of Environmental Protection, Southeast Regional Office, personal communication), but sources of the fecal coliform bacteria had not been identified. Of these sites, only at Muddy Cove was source differentiation of the fecal streptococci bacteria accomplished. At the other locations either fecal streptococci bacteria were not recovered or they were present in very low counts as were the fecal coliform bacteria. Sunset Cove was sampled twice, but on both occasions the counts were low. On these dates an on-shore wind kept the tidal water from receding, so whatever was coming off of the marsh was likely diluted or died off in the "cleaner" sea water.

A description of the findings at Muddy Cove follows.

Muddy Cove

Muddy Cove is a salt water marsh which receives some fresh water input from Gibbs Brook at its northern end. Since 1971, Muddy Cove, Wareham has had a history of restrictions on shellfishing because of fecal contamination. Slightly more than 20 acres were closed from December 1971 until February 1986 when they were reopened for shellfishing. In June of 1987 the same acreage was closed but it was reopened December 1988 (personal communication, Gregg Sawyer, MA Div. of Marine Fisheries). Muddy Cove was sampled for this study on August 11, 1986. Source differentiation of the fecal streptococci was also done.

Gibbs Brook, a freshwater tributary to Muddy Cove, has several possible sources of fecal streptococci including cranberry bogs which provides habitat for birds, insects, small mammals and detrital vegetation and a possible source of human fecal streptococci from a nonsewered trailer park located north of Route 6. Muddy Cove has a few homes surrounding it, plus it receives storm drainage from Route 6, which can receive excrement of dogs, cats, rodents and birds. At the marsh itself, insects, plants, birds and small rodents may all be contributors of fecal streptococci. The birds and small mammals can contribute fecal coliform bacteria.

Birds, such as gulls, are an important contributor of fecal material to Muddy Cove. Large populations of gulls were seen in this area by casusal observers, but were not present during sampling. On August 11, 15.7 percent of the fecal streptococci colonies examined were type group Q from possible avian sources and 35.3 percent were from warmblooded animals.

Stations 1201 through 1204 had elevated fecal counts; the range was 85 to 400 CFU/100 ml. A determination of the actual source of these bacteria was not accomplished. A few houses were located along the edge of the marsh; whether these houses contributed to the closure of this area for shellfishing could not be determined. Other sources of contamination to the Muddy Cove shellfish area may be Gibbs Brook (fecal coliform ranged from 195 to 250 CFU/100 ml), the marsh itself or perhaps the discharge of marine sanitation devices in the Cape Cod Canal or other nearby waters. On this date a slick of what appeared to be macerated fecal material was observed in one of the tidal creeks. This station had a fecal coliform count of 400 CFU/100 ml and a fecal streptococci count of 65 CFU/100 ml. Source differentiation was done on five of the colonies. The results were that three were non-fecal streptococci and two were from warmblooded sources.

Stations 1212, 1205 and 1206 were on Gibbs Brook (see Appendix, Fig. A-15). Sample 1212 was taken just below Dick's Trailer Home Park and had a fecal coliform count of 200 CFU/100 ml. The other fecal indicators at this location were also elevated and suggest warmblooded sources of fecal contaminants. Stations 1205, 1206 were below Route 6 and remained high; 250 and 240 FC CFU/100 ml, respectively.

Correlations were run using SPSSX of log-transformed fecal coliform bacteria with log-transformed fecal streptococci and log-transformed enterococci with log-transformed fecal streptococci (Figure 3, Table A-6). At the salt marsh, only 35.3% of the streptococci were from warmblooded animals yet the correlation of log-transformed enterococci to log-transformed streptococci was high $r = 0.7502$ and significant $p < 0.05$ (see Figure 4). When these results are compared to those from East Branch of Westport River, collected on October 2, 1986, inconsistencies in the correlations and graphs with source differentiation become evident. The section of the Westport River sampled (see Figure A-20) on this date had 90% enterococci (Table A-6). The correlation of log-transformed enterococci to log-transformed streptococci would be expected to be high and significant which it was ($r = 0.8328$, $p < 0.05$).

Since enterococci is a subset of streptococci and is contributed by warmblooded animals, if 90% of the streptococci was found by biochemical differentiation to be from warmblooded animals an r value close to 1 would be expected. If only 35% of the streptococci was from warmblooded animals a lower r value would be expected when the log of enterococci was correlated with log streptococci. Sample variability, the inherent limitation in the data when only 5 fecal streptococci colonies per sample were differentiated, as well as die-off of the indicator organisms in the environment, likely contributed to the failure of this technique to be a good predictor of type of bacterial source impacting an area.

The correlation of log-transformed fecal streptococci with log-transformed fecal coliform bacteria was also positive and strong ($r=0.7261$, $r^2=0.5272$, significance, $p<0.05$ (see Figure 3). This indicated that any increases in fecal streptococci were accompanied by increases in fecal coliform bacteria.

FIGURE 3

REGRESSION—MUDDY COVE,AUGUST 11,1986

LOG STREPTOCOCCI AND LOG FECAL COLIFORM

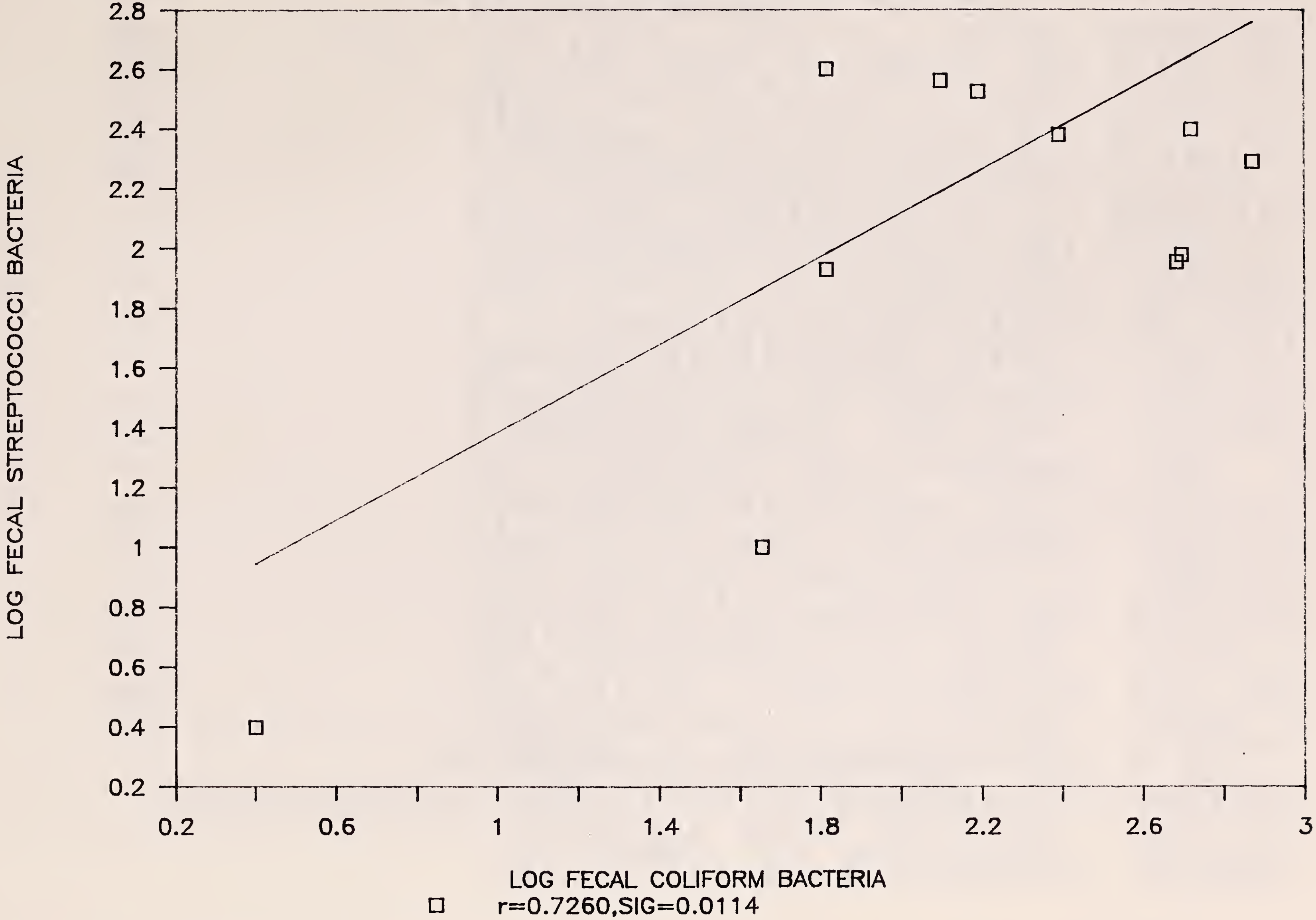
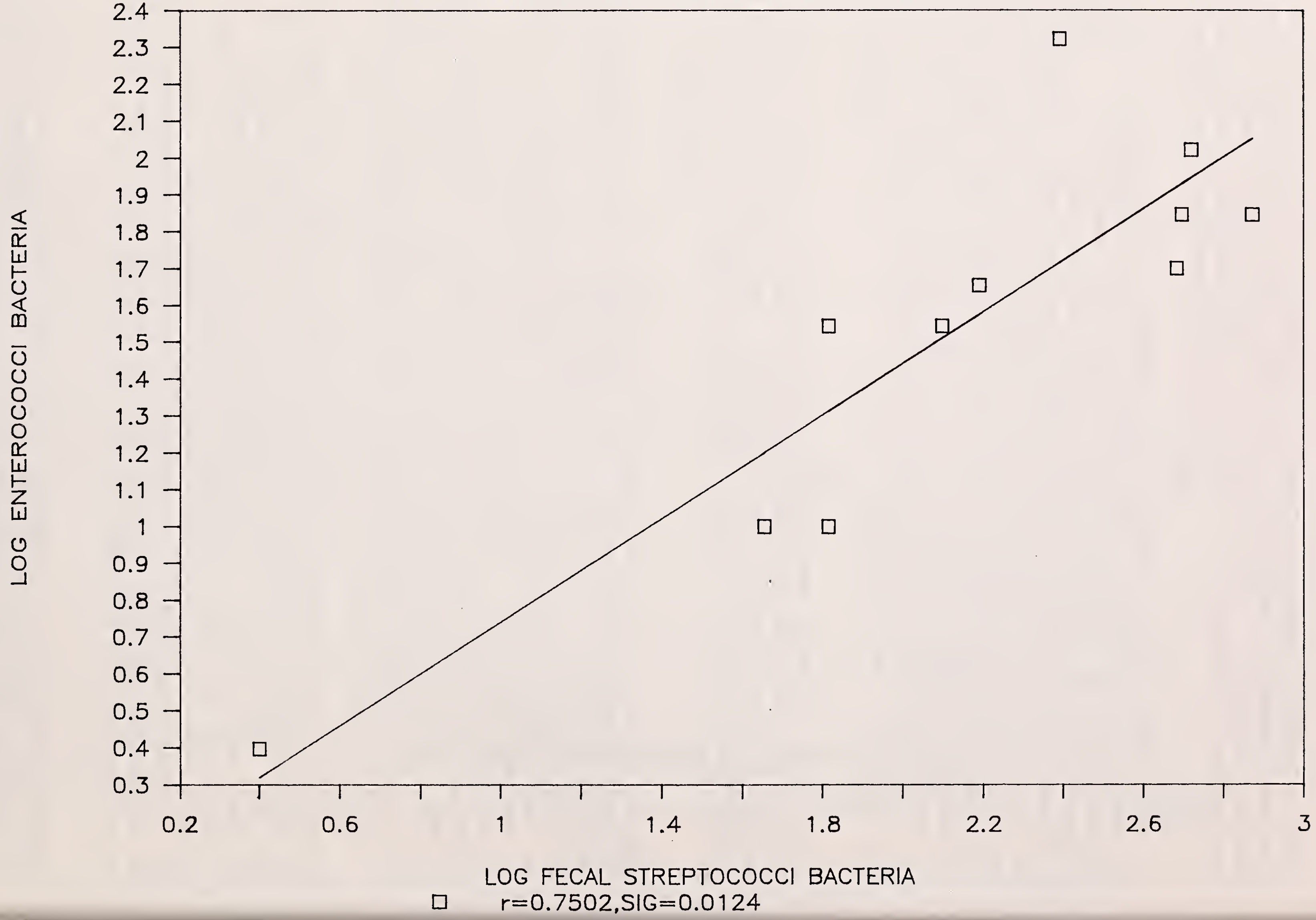


FIGURE 4

REGRESSION—MUDDY COVE,AUGUST 11,1986

LOG ENTEROCOCCI AND LOG STREPTOCOCCI



But the quantities of fecal coliform and fecal streptococci bacteria which are excreted differs from one source to another. The die-off rates of these two groups of indicator bacteria differs as well. So at this salt marsh area with its potential for several types of sources, a less vigorous relationship with lower significance was expected. Because these assumptions were not met, it has been questioned whether the data can be used to identify sources. A larger database for source differentiation of the fecal streptococci bacteria is needed to resolve this issue.

When the log-transformed bacterial data were correlated with physical and chemical parameters (see Table 5), all but the fecal coliform bacteria and E. coli showed significant correlations with chloride. This implies that the marine waters were cleaner and tended to dilute thereby reducing the chemical concentrations and bacterial counts. The fecal streptococci and the enterococci both showed significant negative correlations with chloride and hardness. This same relationship did not exist for the fecal coliform bacteria and the E. coli. As chloride increased the bacterial count did not decrease. This may indicate that multiplication of the fecal coliform and the E. coli bacteria is occurring within the marsh. Recent studies have suggested the possibility of multiplication of fecal coliform and E. coli in marine or estuarine waters (Barnstable County Health and Environmental Department, 1987). This is particularly the case under conditions of high carbon content and protection from sunlight. These conditions are present in the line of vegetative debris (beach wrack) which is located at the high tide mark. The vegetative fragments provide the carbon as well as protection from sunlight to the layers below. Thus, fecal bacteria which may be contributed to the beach wrack by dogs or storm drains, may be retained and even multiply (Barnstable County Health and Environmental Department (1987) within the beach wrack. It is also possible that sources of bacteria located within the marsh such as birds, and small mammals may be contributing enough fecal coliform bacteria to eliminate the dilution effect. On August 11, the lack of correlation of bacteria with chloride suggested that within the marsh some increase in these indicator organisms had occurred. Whether this was by multiplication or via a collection of sources within the marshland could not be determined.

TABLE 5

SIGNIFICANT* CORRELATIONS OF INDICATOR ORGANISMS WITH CHEMICAL AND PHYSICAL PARAMETERS - r VALUES

MARSH, COVE AND BAY SITES - WAREHAM

DATE	pH	TOTAL HARDNESS	SUSP. SOLIDS	DISSOLVED OXYGEN	CHLORIDE	TKN	AMMONIA	TOTAL PHOS.
Onset Bay Aug. 10, 1986								
Enterococci	—	—		—	—	—	—	—
Streptococci	—	—	—	—	—	—	—	—
Fecal Coliform	—	—	—	—	—	—	—	—
Muddy Cove Aug. 11, 1986								
E. coli	—	—	—	—	—	—	—	—
Enterococci	-0.906	-0.715	—	—	-0.822	—	—	—
Streptococci	-0.641	-0.638	—	-0.739	-0.741	—	—	—
Fecal Coliform	—	—	—	—	—	—	—	—
E. coli	-0.812	—	—	—	—	—	—	—
Sunset Cove Aug. 26, 1986								
Enterococci	—	—	—	—	—	—	—	—
Streptococci	—	—	—	—	—	—	—	—
Fecal Coliform		-0.831	—	—	-0.994	-0.707	—	0.899
E. coli	—	—	—	—	—	—	—	—
Sunset Cove Sept. 24, 1986								
Enterococci	—	—	—	—	—	—	—	—
Streptococci	—	—	—	—	—	—	—	—
Fecal Coliform	—	—	—	—	—	—	0.813	—
E. coli	—	—	—	—	—	—	0.795	—

TABLE 5 (CONTINUED)

DATE	pH	TOTAL HARDNESS	SUSP. SOLIDS	DISSOLVED OXYGEN	CHLORIDE	TKN	AMMONIA	TOTAL PHOS.
Broad Cove Oct. 8, 1986								
Enterococci	-0.943	--	--	--	--	--	--	--
Streptococci	--	--	--	--	--	--	--	--
Fecal Coliform	--	--	--	--	--	0.893	--	--
E. coli	-0.988	--	--	--	--	--	--	--

*Correlations were run on all parameters but only those with r values significant at $p<0.05$ were reported

5.0 AGAWAM-WAREHAM RIVERS

The Agawam-Wareham Rivers located in Wareham, were included in this study because of their diverse land-use patterns and because of the closure since 1985 of approximately 290 acres of shellfish beds in this basin (personal communication with Greg Sawyer, Mass. Div. of Marine Fisheries). Commercial harvesting of oysters was permitted here until 1985. With closure, the town lost an important economic resource and Master shellfishermen, a source of livelihood. Given the river's importance, it was hoped that, by source differentiation and other microbial analyses, the sources of pollution to this river system could be identified.

As stated, the land uses along the Agawam-Wareham River are very diverse. These include: cranberry bogs, marshlands, housing areas and marinas. In addition, the Agawam River receives effluent from one of the few wastewater treatment plants in the area, the Wareham Publicly Owned Treatment Works (POTW). The upper part of the Agawam River is agricultural/residential with many cranberry bogs, while downtown Wareham is fairly urbanized with street drains, parking lots, as well as, Warr's Marina. A retaining wall is present in the lower part of the river with pipes leading from street drains. The impact on the water quality from any discharge from these pipes could not be evaluated by this study.

The Agawam-Wareham River was sampled several times during 1986. The first sampling event was on June 23 and began at the Agawam River approximately 400 feet upstream from the first discharge pipe at the Wareham POTW. The survey extended down to the channel marker (number 30) in the lower Wareham River. The purpose of sampling on this date was to try to determine how far down the river the microbial impacts from the Wareham POTW could be detected. It was hoped that the bacterial indicator, Clostridium perfringens could be used for this purpose because it forms long-lived spores. The next step would have been to compare these results with those of another indicator organism, the fecal coliform bacteria, which are known to die-off more quickly in the environment. This plan did not work.

The impact of the treatment plant on water quality could not be distinguished from the many other nonpoint sources of fecal contamination. Neither the fecal coliform nor the Clostridium counts could be used to locate the dominant source to the area. From above the POTW at station 0201 down to 0208, arithmetic means of triplicate fecal coliform samples collected on June 23 (see Table 6) remained at relatively the same density (average 350/100 ml). At station 0209 (the Sandwich Rd. Bridge) the mean went to 458/100 ml; the mean then again increased at the next station 0210 to 496/100 ml. This station is located just downstream of several houses which were constructed on a point of land off of Sandwich Rd. Problems in this area had been documented by DEP - SE Regional Office, Lakeville. Their data appear to suggest a seasonal intermittent source. In 1985, they found the following fecal coliform MPN values:

April 24, 1985	43
May 28, 1985	75
July 23, 1985	350
July 24, 1985	43

TABLE 6

ARITHMETIC MEANS OF TRIPLICATE

BACTERIAL SAMPLES FROM AGAWAM AND WAREHAM RIVERS

JUNE 23, 1986

STATION	FECAL COLIFORM BACTERIA/100 ml	*Escherichia coli*/100 ml	*Clostridium perfringens*/ 100 ml
0201	341	308	5.8
0202	351	291	3.3
0203	323	311	3.3
0204	365	295	2.5
0205	328	273	3.3
0206	310	306	2.5
0207	366	365	7.5
0208	338	310	2.5
0209	458	406	3.3
0210	496	430	5.8
0211	233	216	13.3
0212	53	83	3.3

At station 0211 the next station downstream which is located off undeveloped land approximately 100 feet from shore the fecal coliform mean decreased to 233/100 ml. Finally, at station 0212, the furthest station downstream, the fecal coliform density dropped to a mean of 53/100 ml.

The Clostridium perfringens data could also not be used for its intended purpose. The good removal of the Clostridium spores by the sand filter beds at the POTW resulted in very low mean densities of this potential conservative tracer. On June 23, C. perfringens recovered from stations along the POTW averaged 3.5/100 ml. The mean of the triplicates at station 0201 located above the POTW was 5.8/100 ml. The highest average density was found at station 0211 (13.3/100 ml).

Apparently, sewage which has received some type of secondary treatment can vary considerably in the number of C. perfringens spores that may be found in its effluent. Cabelli (1977) found that the effluent of a sewage treatment plant employing the activated sludge process and chlorination discharged approximately 10,000 C. perfringens/100 ml in the effluent while a secondary plant with sand filtration and chlorination produced an effluent exhibiting 30 C. perfringens/100 ml of sample. No seasonal data were presented so any difference in the performance of sand filter beds during Massachusetts winters could not be determined. Therefore, use of a conservative spore forming bacteria to delineate an impact zone extending from the Wareham POTW was not a viable method.

Because the elevated bacterial counts were found upstream of the POTW, additional sampling was conducted in upstream areas on June 30. Sampling on this date provided no answers as to the source of the elevated bacterial counts in the lower part of the Agawam River. The outlet to Mill Pond located upstream of the POTW had a fecal coliform count of <10 CFU/100 ml. This station (0301) is physically separated from the others by a small weir located below the POTW. Thus, no exchange of tidal waters could have occurred here as was possible at station 0201.

Fecal coliform densities of 5600 CFU/100 ml and 5000 CFU/100 ml were observed in duplicate samples collected at station 0303 at the outlet of Glen Charlie Pond. This station was located at the downstream side of a dirt road off Glen Charlie Road. Additional work should be done in this area to determine what the source of this recent pollution could be. This sample was collected in an area where the possibility exists that the water was contaminated after it left the pond. The outlet water was only flowing at a trickle and there was no indication of a likely bacterial source.

On July 7,9, and 21, 1986 an attempt was made to do source differentiation work in the Wareham River estuary. On August 3 Warr's Marina in the Wareham estuary was sampled. Discussion of these results is included in Section 8.0.

On July 7, the fecal coliform counts in the estuary stations, which extended from below Warr's Marina down to Broad Marsh River (see Figure A-5), ranged from <10 to 20 CFU/100 ml and the fecal streptococci <10 to 20 CFU/100 ml as well. This did not provide sufficient fecal streptococci colonies for source differentiation, nor did it provide bacterial counts high enough to plot density gradients. On this date an effort was made to establish a gradient of bacterial densities and nutrient concentrations to aid in locating impact zones from nonpoint sources of pollution. A concentration gradient for ammonia from the shore to a location 50-feet from the shore was evident in three out of four sampling areas, all of which were located in front of houses. Since there was no accompanying increase in total Kjeldahl nitrogen or total phosphorus it is suggested that this may have been contributed by septic system leaching fields. A similar situation is described in Section 7.0.

The Wareham River estuary was again sampled on July 9, 1986 in a second attempt to isolate fecal streptococci colonies for source differentiation. Again an on shore wind kept the cleaner tidal waters from receding, thus keeping the bacterial counts relatively low. Even with the comparatively low bacterial counts the arithmetic mean of the fecal coliform MPN was 62.9/100 ml which exceeds the value for approved shellfish areas (i.e., 14 MPN/100 ML).

The same area, although not the same stations (see Figure A-8) was sampled on July 21. The range of the fecal coliform values was 80 to 320 CFU/100 ml and the range for fecal streptococci 35 to 185 CFU/100. Sufficient colonies of fecal streptococci were isolated for source differentiation which indicated that 45% of 58 colonies were non-fecal streptococci, 2% Group Q, or tentative S. avium, from birds, 48% warmblooded, 0% livestock, insects, and 0% vegetation. Although the high incidence of false positives caused some problems in the evaluation of these data, the high percentage of warmblooded sources was evident.

Significant correlations of the bacterial indicators with various chemical and physical parameters are shown in Table 7. On July 21, when source differentiation was done, a significant correlation was not found between log-transformed streptococci and log-transformed fecal coliform (Figure 5). However, the regression of log-transformed enterococci with log-transformed streptococci (Figure 6) was significant and positively correlated r= 0.7974, significant P <0.05. This might be expected since warmblooded animals, which contribute the enterococci, was the source of 48% of the fecal streptococci on this date (Table A-6).

From these data it can not be definitively established whether the source of the warmblooded fecal streptococci is from humans or other mammals such as dogs and cats. In the fecal matter of warmblooded animals pathogens as well as the normal enteric population can be present. When humans are the fecal source to an area any pathogens present represent a definite threat to public health if they are ingested via the consumption of shellfish or ingested while swimming. If excrement from other warmblooded animals is impacting an area via nonpoint sources the degree of risk is not as well quantified as with human pathogens. Viruses, in particular, are host specific and are not typically transferred from one species to another. Some risk is still present; however, of contracting bacterial skin diseases or bacterial gastrointestinal illness from warmblooded animals other than humans. Thus, there is a need for control of point sources and nonpoint sources of fecal contamination as well in this area. Likely sources of fecal contamination from warmblooded animals includes Warr's Marina, septic systems on eastern shore of Wareham River and storm drains which collect runoff from downtown Wareham.

TABLE 7

SIGNIFICANT* CORRELATIONS OF INDICATOR ORGANISMS WITH

CHEMICAL AND PHYSICAL PARAMETERS - r VALUES

WAREHAM RIVER ESTUARY

DATE	pH	TOTAL HARDNESS	SUSP. SOLIDS	SPEC. COND.	TKN	AMMONIA	TOTAL PHOS.	DISSOLVED OXYGEN
July 7, 1986								
Enterococci	--	--	--	--	--	--	--	--
Streptococci	--	--	0.855	--	--	--	0.901	--
Fecal Coliform	--	--	--	--	--	--	--	--
E. coli	--	--	--	--	--	--	--	--
July 9, 1986								
Enterococci	--	--	--	--	--	--	--	--
Streptococci	--	--	--	--	--	--	--	--
Fecal Coliform	--	--	--	--	--	--	--	-0.842
E. coli	--	--	--	--	--	--	--	--
July 21, 1986								
Enterococci	--	-0.755	-0.606	--	--	--	--	-0.580
Streptococci	--	-0.718	--	--	--	--	--	--
Fecal Coliform	--	-0.650	-0.584	--	--	--	--	-0.632
E. coli	--	-0.699	-0.603	--	--	--	--	-0.616

TABLE 7 (CONTINUED)

DATE	pH	TOTAL HARDNESS	SUSP. SOLIDS	SPEC. COND.	TKN	AMMONIA	TOTAL PHOS.	DISSOLVED OXYGEN
Aug. 3, 1986								
Enterococci	—	—	—	—	—	—	—	—
Streptococci	—	—	—	—	—	—	—	—
Fecal Coliform	—	—	—	—	—	—	—	—
E. coli	—	—	—	—	—	—	—	—

* Correlations were run on all parameters but only those with r values significant at $p<0.05$ were reported.

FIGURE 5

REGRESSION—WAREHAM RIVER,JULY 21,1986

LOG STREPTOCOCCI AND LOG FECAL COLIFORM

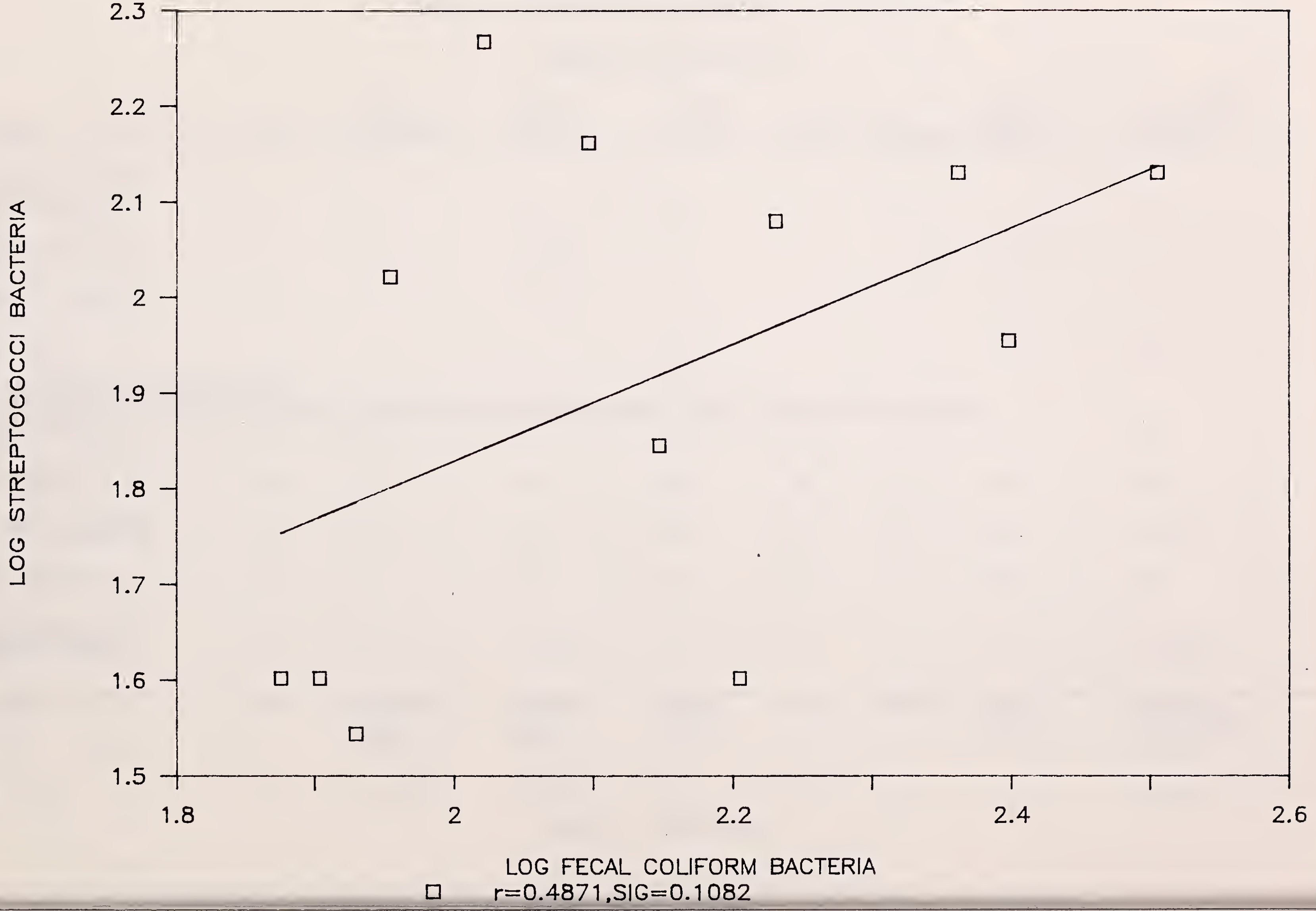
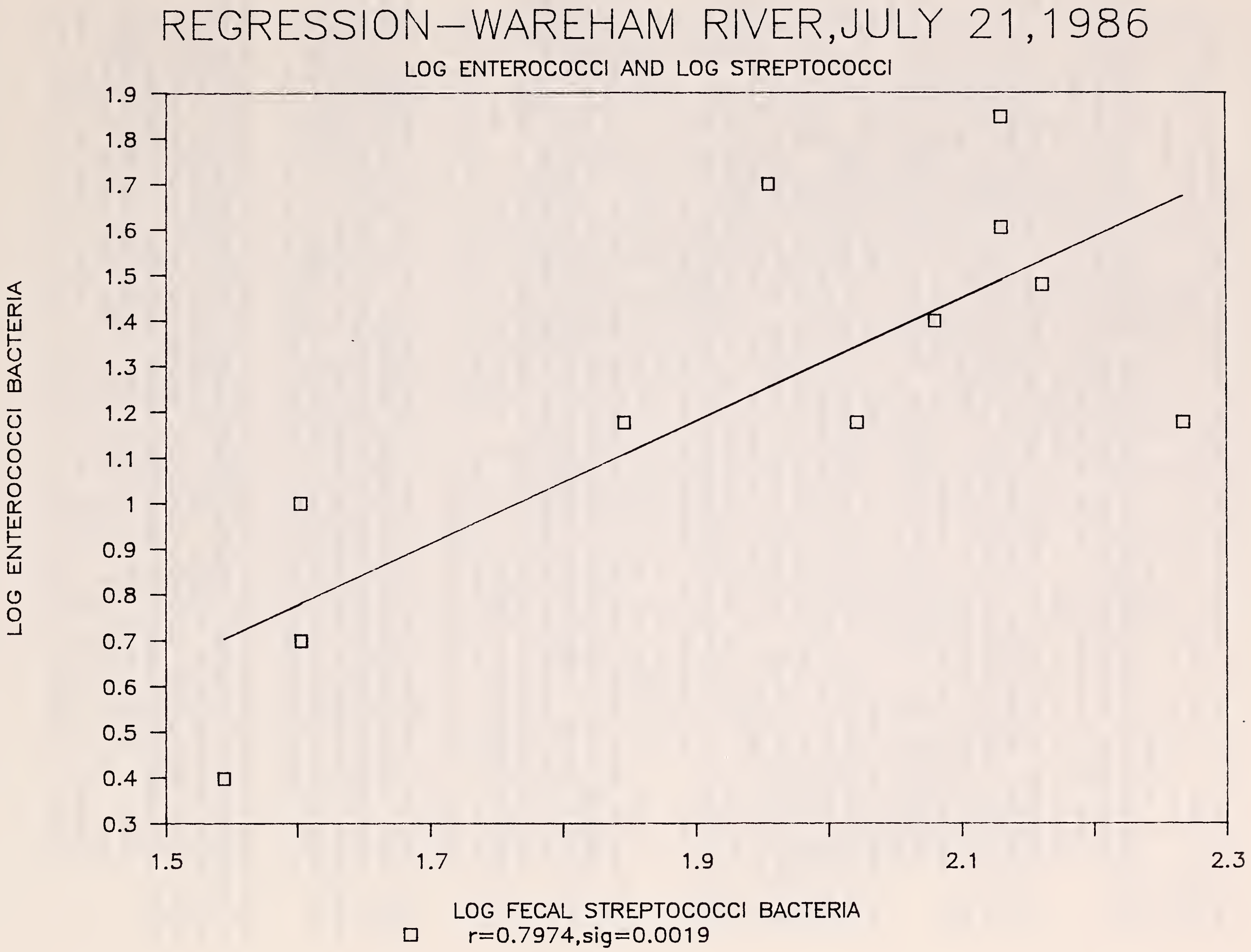


FIGURE 6


REGRESSION—WAREHAM RIVER,JULY 21,1986
LOG ENTEROCOCCI AND LOG STREPTOCOCCI
LOG ENTEROCOCCI BACTERIA
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
1.5
1.7
1.9
2.1
2.3
LOG FECAL STREPTOCOCCI BACTERIA
□ r=0.7974,sig=0.0019

6.0 WESTPORT RIVER - AGRICULTURAL AREA

The East Branch of the Westport River flows through a scenic agricultural area in the town of Westport. Corn, cattle, pig and chicken farms are located both along the river and its major tributaries. The specific area of concern extends from the Head of the Westport to Hix Bridge and is closed to shellfishing because of elevated bacterial levels in overlying water. Severe problems of bacterial contamination in this area of the Westport River had already been documented (Kelly et al., 1986, Westport River Rural Clean Water Program, 1983) before this study. This was an attempt to determine that agricultural runoff from livestock and not illegal tie-ins of human waste were responsible for the elevated bacterial counts in the East Branch of the Westport River. According to the Westport River Watershed Committee Report in 1983 there were 32 active dairy and beef cattle farms along the East Branch of the Westport River which had a total cow population of 3900. Fecal coliform contamination is significant and widespread in the Westport River from Head of the Westport to Hix Bridge the area where the farms are located. Although livestock and their excrement was in evidence in the sampling areas, _S_. _bovis_ and _S_. _equinus_, which are indicative of livestock fecal waste were not isolated. These two indicators die-off quickly in the environment (Clausen et al., 1977). This creates difficulties in isolating them from waterbodies which receive fecal waste from livestock on an intermittent basis.

In 1986, KF medium was used for the recovery of streptococci bacteria. This medium was chosen because of its reported good recovery of _S_. _bovis_ and _S_. _equinus_ (e.g., Geldreich et al., 1964, Pavlova et al., 1972, Kenner et al., 1960). Since _S_. _bovis_ and _S_. _equinus_ were not isolated where the likelihood of their being present was high, a different streptococci medium (PSE) was used in 1987. Again in these agricultural areas _S_. _bovis_ and _S_. _equinus_ were not recovered (Beskenis, 1987).

Westport River Above Hix Bridge

Sampling in the agricultural area of the East Branch of the Westport River was done five times during the summer of 1986. On two of these occasions (September 17, October 2) source differentiation of the fecal streptococci was done. On each of the sampling dates rain was either falling or had fallen within the previous three days. The amount of rainfall ranged from 1.67 to 0.11 inches as measured by N.O.A.A. at New Bedford.

Fecal contamination to this part of the river is believed to be chiefly contributed by runoff from agricultural land. The amount of previous rainfall greatly affects the results. Only 0.11 inches of rainfall fell previous to the July 28 sampling and the bacterial counts below Jessie's Neck Cove were significantly lower than on other dates when the precipitation was much higher. Snell Creek, which collects the runoff from a lot of agricultural land, runs into the cove at Jessie's Neck. The low average bacterial counts (average 112 FC/100 ml) found on this date below Jessie's Neck were also present in samples collected along a transect across the river (Table A-15).

Samples collected August 4 from a transect originating from Jessie's Neck had much higher densities (Table 8). On this date 0.64 inches fell. Tidal effects quickly reduced this density so that the first station sampled south of Hix Bridge had a fecal coliform count of <20/100 ml. If there's a lot of freshwater runoff the "plug" of fecally contaminated material extends further downstream. On July 14 when there had been 1.67 inches of precipitation the average fecal coliform density just south of Hix Bridge was 2300/100 ml.

Everett Cove located upstream of Jessie's Neck on the eastern shore is also significantly impacted by agricultural runoff. On July 14 when 1.73 inches of rain fell a sample collected at the head of the cove had 50,000 FC/100 ml and the mouth 80,000 FC/100 ml (Table 9). While on September 17 when 0.73 inches fell the fecal coliform density at the mouth of the cove was 2900/100 ml. The variation in rainfall was likely a contributing factor to this difference.

The bacterial densities of the indicator organisms help in determining sources of fecal contamination. But, identifying bacterial sources by their fecal streptococci components has not been shown to be as reliable. On two dates source differentiation of the fecal streptococci was done on samples from the agricultural part of the Westport River. However, on neither date were S. bovis and S. equinus isolated. These two streptococci are most indicative of fecal contributions from livestock and the failure to isolate them here is a factor in not recommending this method for further use. The source differentiation scheme did indicate that fecal contributions were dominated by warmblooded animals and livestock are included in this grouping.

On September 17, 1986 source differentiation of fecal streptococci was done on samples collected above Hix Bridge. Source differentiation totals were: 75 percent from warmblooded animals and 22.9 percent Group Q and 2.1 percent non-fecal streptococci (Table A-6). When log-transformed enterococci was correlated with log-transformed streptococci a significant correlation of $p < 0.05$ and a strong correlation of $r = 0.7685$ was found (Figure 8). Log-transformed streptococci versus log-transformed fecal coliform bacteria was also significant $p < 0.05$, $r = 0.7794$ (Figure 7).

On October 2, the log streptococci and log fecal coliform were strongly correlated with $r = 0.7665$, significance $p<0.05$ (Figure 9). The log-transformed streptococci and log-transformed enterococci were also correlated $r = 0.6936$, significance $p < 0.05$ (Figure 10). Source differentiation on this date showed 90 percent of the fecal streptococci were from warmblooded animals.

The predominance of the fresh water sources is evident in correlations between chemical parameters with log-transformed bacterial indicator data collected on August 4, September 17 and October 2. The negative chloride correlation with these bacterial indicators can be interpreted to mean that as chloride concentration increases the bacterial counts decrease (see Table 10) which suggests a freshwater source.

TABLE 8

FECAL COLIFORM DENSITIES (per 100 ml) EXTENDING ACROSS THE WESTPORT RIVER FROM JESSIE'S NECK TO THE OPPOSITE SHORE

JULY 28, 1986

	LOCATIONS					
DATE	A	B	C	D	E	F
7/28* 1986	112	97	52	12	12	25
8/4 1986	2400	NS	2100	NS	NS	4000

* Average values
NS not sampled

A - approximately 30' from western shore
B - approximately 150' from western shore
C - mid-channel
D - approximately 300' from eastern shore
E - approximately 200' from eastern shore
F - approximately 40' from eastern shore

TABLE 9

SELECTED FECAL COLIFORM DENSITIES (per 100 ml) FROM STATIONS LOCATED IN THE AGRICULTURAL AREA OF THE EAST BRANCH OF THE WESTPORT RIVER

Rainfall** (inches)	Date	Jessie's Neck Cove	Everett Cove	Along Farm at Peligs Point	Drainage Ditch at Peligs Point	South of Hix Bridge
1.67	7/14/86	4300	50,000(A) 80,000(B)	1840	2250	2300
0.11	7/28/86	avg. 112*	—	—	—	10
0.64	8/4/86	2400	—	—	—	—
0.73	9/17/86	900(C) 90(D)	2900(B)	—	—	—

* Sampled approximately 30 feet from shore just downstream of the mouth of the cove.

** Rainfall was measured and recorded at the nearest NOAA station, New Bedford.

A - head of Everett Cove
B - mouth of Everett Cove
C - head of Jessie's Neck Cove
D - mouth of Jessie's Neck Cove

FIGURE 7

REGRESSION WESTPORT RIVER—SEPT.17,1986

FECAL STREPTOCOCCI AND FECAL COLIFORM

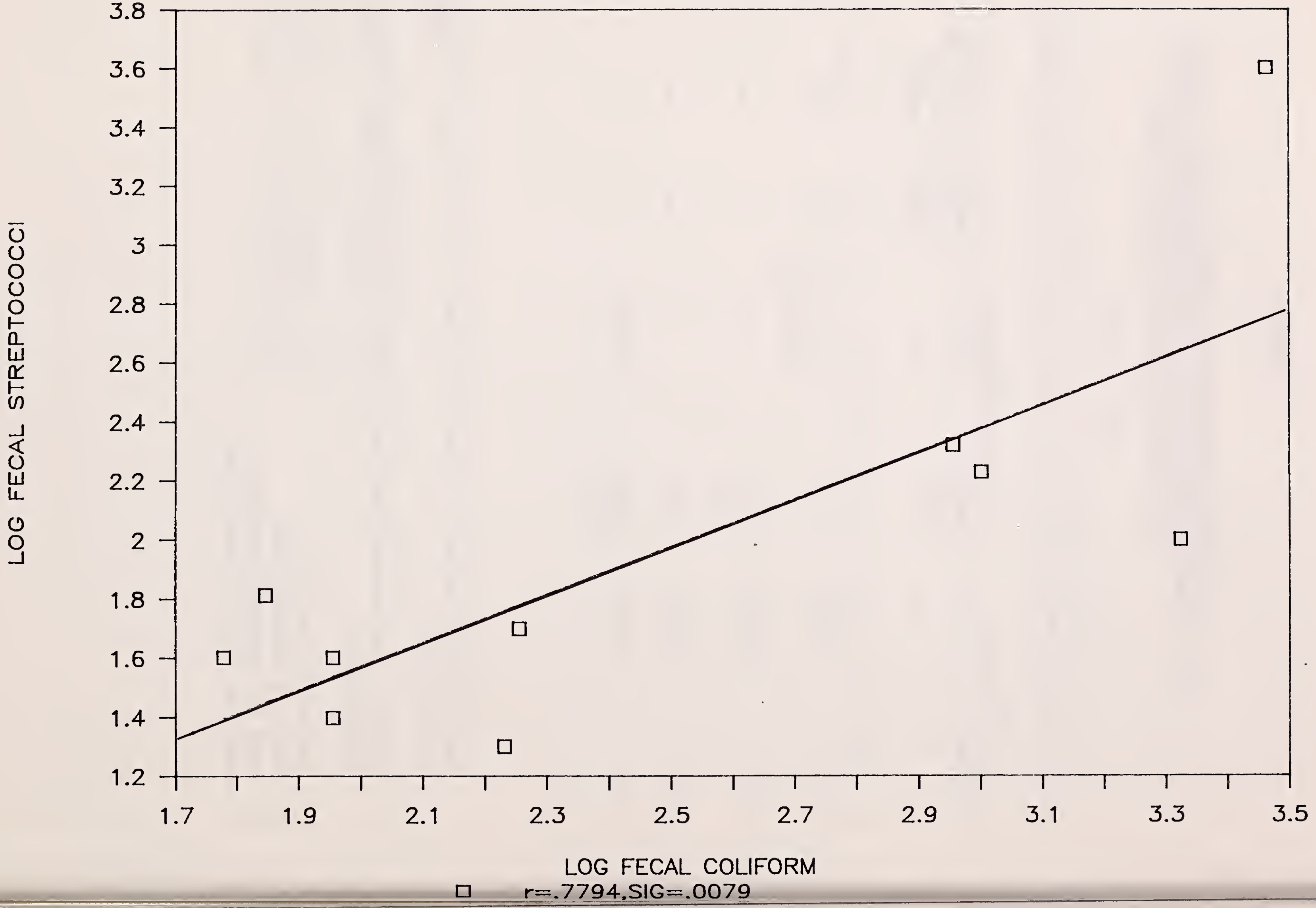


FIGURE 8

REGRESSION WESTPORT RIVER—SEPT.17,1986

LOG ENTEROCOCCI AND LOG STREPTOCOCCI

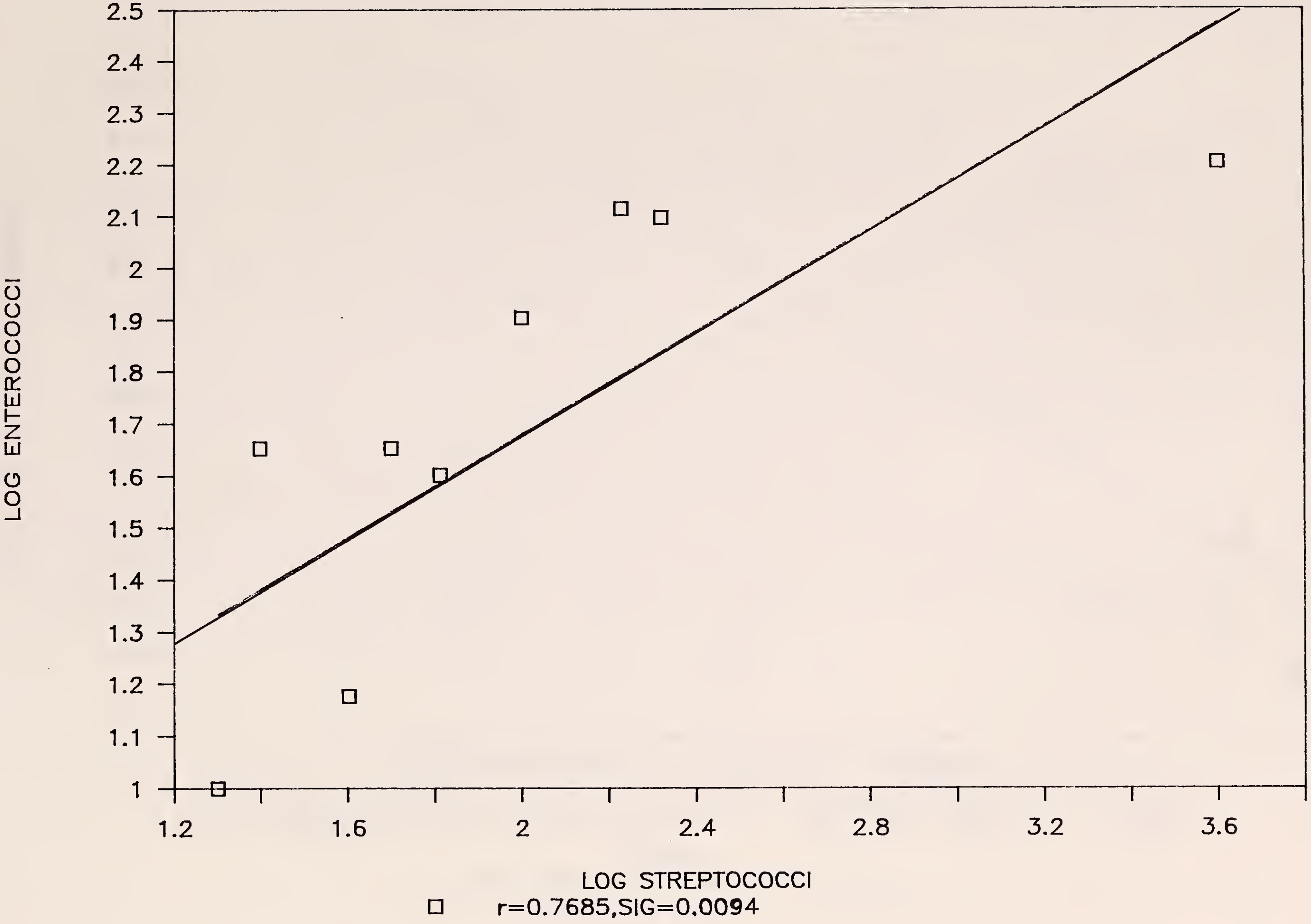


FIGURE 9

REGRESSION—WESTPORT RIVER,OCT.2,1986

LOG STREPTOCOCCI AND LOG FECAL COLIFORM

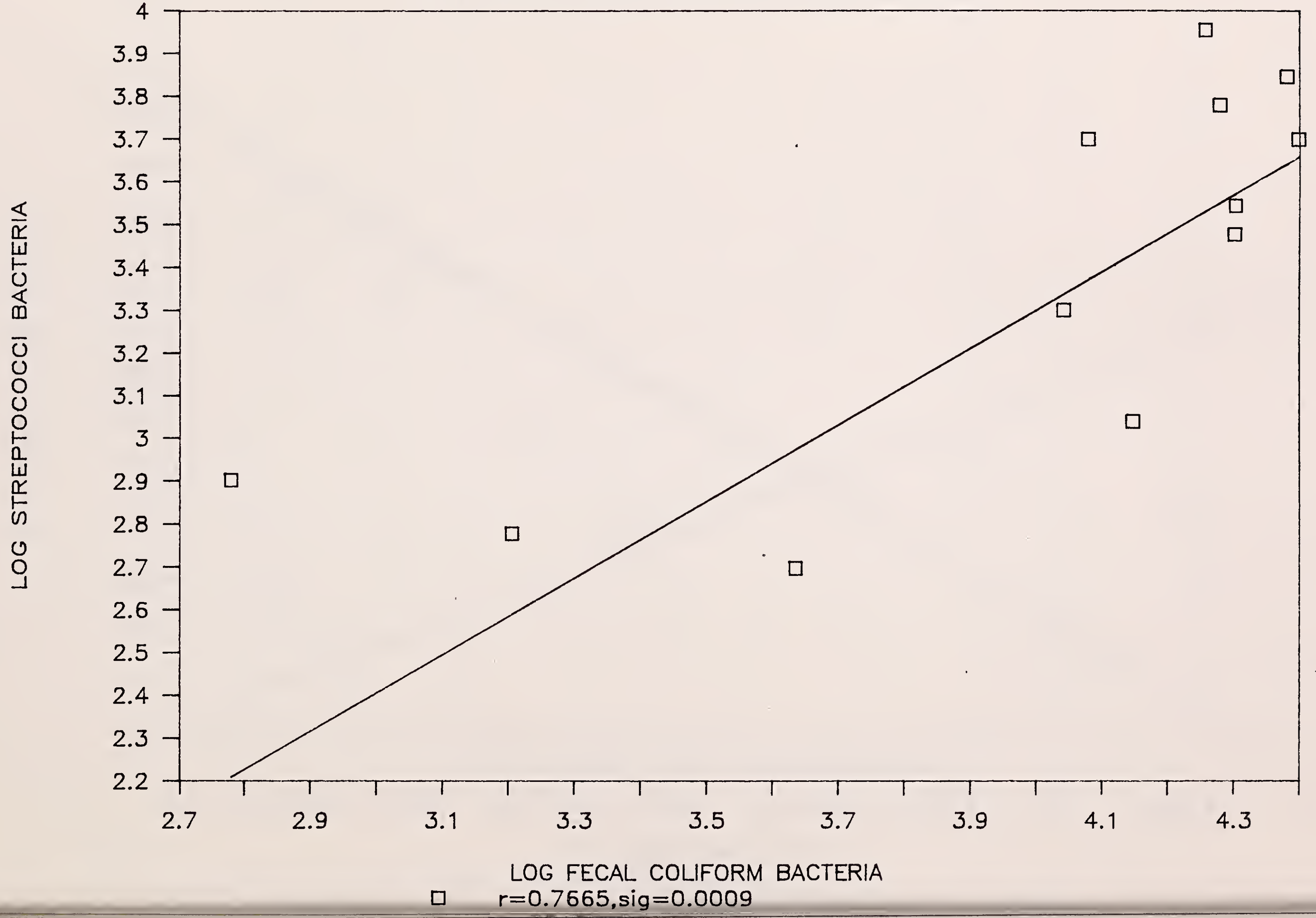


FIGURE 10

REGRESSION—WESTPORT RIVER,OCT.2,1986

ENTEROCOCCI AND FECAL STREPTOCOCCI

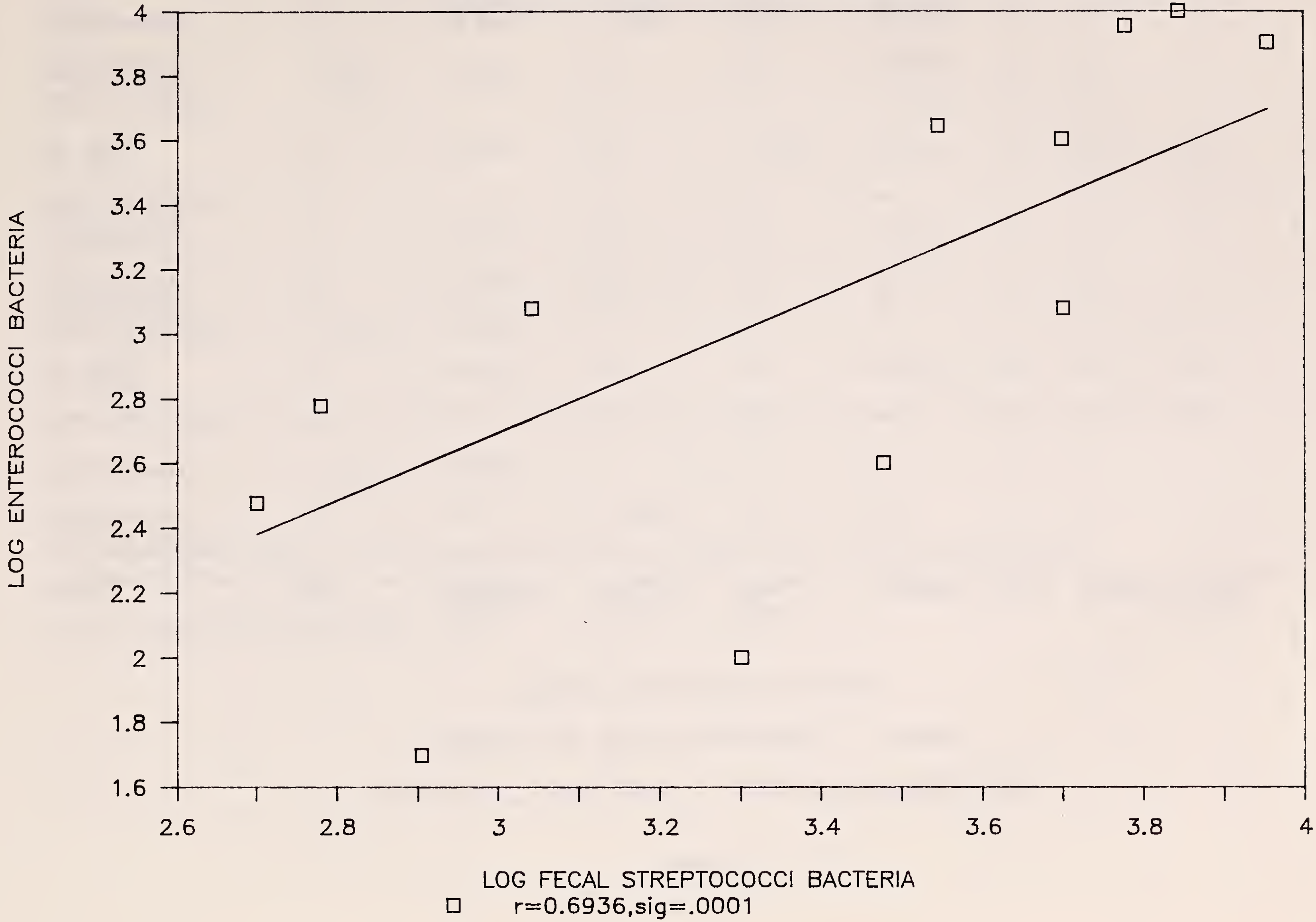


TABLE 10

SIGNIFICANT* CORRELATIONS OF INDICATOR ORGANISMS WITH CHEMICAL AND PHYSICAL PARAMETERS - r VALUES

WESTPORT RIVER ABOVE HIX BRIDGE

DATE	pH	TOTAL HARDNESS	SUSP. SOLIDS	SPEC. COND.	CHLORIDE	TKN	AMMONIA	TOT PHOS
July 14, 1986								
Enterococci	—	—	-0.804	—	**	—	—	—
Streptococci	-0.734	-0.833	—	—	**	—	—	—
Fecal Coliform	—	—	—	—	**	—	—	—
E. coli	—	—	—	—	**	—	—	—
July 28, 1986								
Enterococci	—	—	—	—	**	—	—	—
Streptococci	—	—	—	—	**	—	—	—
Fecal Coliform	—	—	—	—	**	—	—	—
E. coli	—	-0.764	—	-0.882	**	—	—	—
Aug. 4, 1986								
Enterococci	-0.840	-0.882	—	—	-0.834	—	—	—
Streptococci	—	-0.953	0.966	—	-0.986	—	—	—
Fecal Coliform	—	—	0.857	—	**	—	—	—
E. coli	—	—	0.878	—	-0.812	—	—	—
Sept. 17, 1986								
Enterococci	-0.747	—	—	—	-0.722	—	—	—

TABLE 10 (CONTINUED)

DATE	pH	TOTAL HARDNESS	SUSP. SOLIDS	SPEC. COND.	CHLORIDE	TKN	AMMONIA	TOT PHOS
Streptococci	-0.716	--	--	--	-0.749	--	--	--
Fecal Coliform	-0.755	--	--	--	-0.851	--	--	--
E. coli	--	--	--	--	-0.731	--	--	--
Oct. 2, 1986 Enterococci	--	-0.724	--	--	-0.735	--	--	--
Streptococci	--	-0.868	--	--	-0.890	--	--	--
Fecal Coliform	-0.795	-0.846	--	--	**	--	--	--
E. coli	--	-0.686	--	--	-0.686	--	--	--

* Correlations were run on all parameters but only those with r values significant at $p<0.05$ were reported.

** Not sampled on these dates

7.0 WESTPORT RIVER - RESIDENTIAL AREAS

The state code 310 CMR 15.00 entitled: Minimum Requirements for the Subsurface Disposal of Sanitary Sewage Environmental Code, Title V (referred to as Title V), stipulates the setback requirements for septic system leaching fields from both public and private wells and adjacent waterbodies. Currently, a leaching field must be 50 feet from a waterbody to prevent the possibility of impacts on the water quality. But, is this setback requirement sufficient to protect water quality? What about areas where the setback requirement is not met; is there a measurable reduction in water quality based on coliform bacteria and nutrients? To evaluate these questions more needs to be learned about the critical factors affecting bacterial and nutrient transport from leaching fields. Factors such as housing density, soil type, age of septic systems, and ground water levels should all be examined for their relative contributions to the problem.

Some of these factors have been examined by George Heufelder (Barnstable County Health Department) and Peter Wyschall (Boston University) for the Buzzards Bay Program (in press). There are several areas in Buzzards Bay either closed to shellfishing or threatened with closure where identifiable sources, other than houses along the shore, are not evident. The purpose for sampling in some of these areas was to try to establish a link between housing areas and bacterial and nutrient inputs to the adjacent water body. Because these are tidal areas, samples were collected two hours after high tide to try to assess "worst-case" conditions. Additional work remains to be done to establish if this time frame actually represents "worst-case" conditions.

Several areas were chosen for study. These included Broad Cove and East River, Wareham (October 8, 1986), Sunset Cove, Wareham (August 26 and September 24) and the lower segment of the Westport River sampled on August 4 and August 19, 1986. Only at the Westport River was there any quantifiable amount of fecal bacteria present.

Westport River Below Hix Bridge

Sites below Hix Bridge on the east bank of East Branch of the Westport River were chosen for study because of their isolated areas of dense housing. Also, those houses on the west side of the river are in apparent compliance with current Title V regulations while on the opposite shore many are not. Jim Walsh, Health Officer, for the town of Westport, had also communicated to DWPC personnel that problem septic systems existed on the east side of the river (personal communication). However, despite sampling during what was believed to be "worst-case" conditions (i.e., approximately two hours after high tide and sampling close to shore) no apparent increase in fecal indicators could be attributed to these houses. This was not totally surprising. Studies have shown that, although bacteria will be transported through wetted soils and will move in the direction of ground water flow (Romero, 1970), processes acting upon them will limit their travel range. Clogging of the soil pores may limit bacterial movement as can environmental changes in parameters such as temperature, and oxygen levels, which can result in die-off of the indicator bacteria. Baars (1957) examined the movement of bacteria from large sewage infiltration basins in generally sandy soil, and found that the bacteria were adsorbed by soil particles within the first 10 feet of travel. If this is so then the Merrimac - Hinckley soils which are present in this area and are also sandy and highly permeable should also be able to retain bacteria. Kelly et al (1986) suggest that adequate filtration of septic system waste may not occur in these soils.

The U.S. Environmental Protection Agency (1975) also had a study to evaluate the impact of septic systems on shellfish areas. Their study showed that the direct transport of fecal coliform bacteria from septic tanks into canals at Surf City, North Carolina accounted for an increase of less than 3 MPN/100 ml for both total and fecal coliform bacteria. Runoff, however, accounted for 330 to 13,000 fecal coliform bacteria/100 ml to shellfish waters. If the septic systems in the vicinity of the Westport River only account for a similar increase of 3 MPN over background levels, then the type of sampling done for this study and for most monitoring surveys would be inadequate to be able to determine this slight increase and indeed it would be impractical to do so.

In contrast stormwater runoff in this area can cause a dramatic increase in fecal coliform densities. On August 16, Hurricane Charley swept through the Westport area and deposited 1.11 inches of rain. The runoff from this storm resulted in the highest bacterial densities recorded below Hix Bridge (range FC 45 to 1100 MPN/100 ml) during the study. Unfortunately no stations were sampled upstream of Hix Bridge on this day. However, the first station (1301) which was sampled because of its location above the housing area on the western side, had 1000 FC/100 ml. The next two stations (1303, 1304) located downstream of two houses in apparent compliance of setback requirements from surface water bodies and one located approximately 20 feet from shore, had 1100 FC/100 ml. Station 1306 located south of the first group of houses and opposite a wooded area had 600 FC/100 ml. It is believed that runoff from this storm pushed a "plug" of fecally contaminated water from the agricultural land upstream farther downstream than on other sampling occasions.

After the August 16 storm, stations on the eastern side of the river were also sampled. Houses on this side of the river are located close to shore. The bacterial counts here ranged from 45 to 90 FC/100 ml. Dilution continued to reduce the fecal coliform densities downstream, but at the same stations nutrient concentrations fluctuated regardless of dilution. For stations 1309 to 1312 chloride remained the same while the ammonia concentration fluctuated from 0.08 mg/l to 0.20 mg/l. Also, when regressions were calculated (Table 11) on log-transformed data, negative correlations were found with total hardness and chloride for enterococci, streptococci and fecal coliform while ammonia had a positive correlation with fecal coliform and with fecal streptococci ($r=0.727$, significance $p<0.05$ and $r=0.795$, significance $p<0.05$, respectively). Sources other than septic leachate cannot be ruled out for the ammonia but this phenomenon occurred at a time when the water table was elevated and in an area where many septic systems could have been flooded as a result of the storm.

TABLE 11

SIGNIFICANT* CORRELATIONS OF INDICATOR ORGANISMS WITH CHEMICAL AND PHYSICAL PARAMETERS - r VALUES

WESTPORT RIVER BELOW HIX BRIDGE

DATE	pH	TOTAL HARDNESS	SUSP. SOLIDS	SPEC. COND.	CHLORIDE	TKN	AMMONIA	TOTAL PHOS.
Aug. 4, 1986								
Enterococci	--	--	--	--	--	--	--	--
Streptococci	--	-0.981	--	--	--	--	--	--
Fecal Coliform	--	--	--	--	--	--	--	--
E. coli	--	--	--	--	--	--	--	--
Aug. 19, 1986								
Enterococci	--	-0.735	--	--	-0.941	--	--	--
Streptococci	--	-0.667	--	--	-0.710	--	0.795	--
Fecal Coliform	--	-0.703	--	--	-0.922	--	0.727	--
E. coli	--	--	--	--	-0.648	-0.662	--	--

* Correlations were run on all parameters but only those with r values significant at $p<0.05$ were reported.

8.0 COASTAL MARINAS

Coastal marinas offer many social and economic benefits to an area and to the boating community in general. However, they can also cause some adverse impacts. A poorly situated marina can contribute to degraded water quality (US EPA, 1985). Serious public health and water quality problems can quickly arise if boat owners discharge untreated fecal waste into the adjacent waters. Areas with poor circulation can quickly exceed the 14 FC/100 ml standard for approved shellfish areas.

In recognition of this problem the Interstate Shellfish Sanitation Conference in 1988 adopted new regulations to establish prohibited, restricted or conditionally approved shellfish areas within a marina and if necessary "beyond the marina proper." The extent of the zone of protection will be unique for each marina based in part upon dilution characteristics, bacterial die-off and marina usage.

One way to reduce the bacterial loading which can occur at marinas is to require the installation and use of pump-out facilities. Currently, the Massachusetts Division of Water Pollution Control does have some authority to require that pump-out facilities be provided under Massachusetts General Laws, Chapter 91 Sec. 59B passed in 1970 which states the following: "No marina shall be operated without a license issued by the Division of Water Pollution Control. Said Division shall not issue any such license unless such marina provides adequate facilities for the collection, treatment and disposal of sewage or other sanitary wastes."

At Buzzards Bay two marinas in Wareham were chosen to determine if bacterial problems exist at these areas. Warr's marina, located on the Wareham River, offers a pump-out facility for its customers use. This area was sampled on August 3, 1986. On August 10, 1986, Point Independence Yacht Club and Onset Bay were sampled. At the time of the sampling neither of these marinas had pump-out facilities.

Regulations requiring the design, construction, installation and operation of marine sanitation devices are under the jurisdiction of the U.S. Coast Guard. They also have authority for enforcement (Coast Guard DOT S159, CFR33 CN1 (7-1-86). There are three types of marine sanitation devices currently being installed on boats with Coast Guard approval and they provide different levels of treatment. These are described in a publication of the Southeastern Regional Planning and Economic Development District (1981). The U.S. Environmental Protection Agency established standards for the use of marine sanitation devices; these are presented in the Clean Water Act of 1987, Section 312 Marine Sanitation Devices. If a boat has an installed marine head it must be equipped with a U.S. Coast Guard approved Type I, Type II, or Type III marine sanitation device (see Table 12).

If pump-out facilities are constructed, would all boaters be required to use them and would they have an impact on the water quality surrounding marinas? Not all waste is suitable for pump-out facilities. Type III systems are simple holding tanks for fecal waste and should only be emptied into a pump-out facility when in shore or pumped out into the receiving water when a boat is outside the three mile limit. Unfortunately, principally because of the odor

TABLE 12

DESCRIPTION OF TYPES OF MARINE SANITATION DEVICES*

TYPE	DESCRIPTION	DISINFECT	ALLOWABLE DISCHARGE OF BACTERIA (FC/100 ml)	ALLOWABLE DISCHARGE OF TOTAL SUSPENDED SOLIDS (mg/l)	DISCHARGE WATERBODY INSHORE	STILL BEING INSTALLED OR USED
I	Macerator/ chlorinator	Yes	1000	No visible floating solids	Yes	Had to be installed by 1980
II	Physical/chemical or Biological Treatment	Yes	$\leq$200	<150	Yes	Yes
III	Recirculation Systems	Yes	$\leq$200	<150	Yes	Yes
	Incinerators	Yes	--	<150	Yes	Yes
	Holding Tanks	No	--	--	No must be 3 miles off shore	Yes

* Based on: Southeastern Regional Planning and Development District, 1981.

problems, Type III systems are not the most commonly installed marine sanitation device being installed on new boats.

Type I sanitation devices receive some disinfection but they do not meet current wastewater discharge standards. They are no longer being installed on boats so their importance will continually decrease.

Type II systems do meet wastewater discharge standards of 200 FC/100 ml. But after treatment with formalin or similar compounds to disinfect and to control odors, the material is no longer suitable for discharge to the ground via septic systems nor to sewage treatment plants. This is because of the formalin which in high concentrations could disturb the microbial degradation of wastes. Some Type II systems disinfect with chlorine, but this substance is known to cause toxicity problems as well.

Although Type II systems should be able to meet water quality standards, this is not always the case. If a system is improperly used, as can occur if the boat owner stops disinfecting, then the boater will be discharging untreated waste which would be the same as a Type III system. Type II material, because of the disinfectants used, is not typically suitable for in-ground sewage disposal or for sewage treatment plants.

Results From Marinas

Warr's Marina was sampled on August 3. The weather on this morning was overcast with a forecast for rain in the afternoon. This likely kept many people from coming to their boats, but some were present having slept on their boats. DWPC sampling found the range of the fecal coliform bacteria from above, within and below the marina, to be <20 to 12,000 CFU/100 ml. Although this marina does have a pump-out facility, a boat owner discharged his toilet while the area was being sampled. He had a Type I macerator/chlorinator system but at the time of the release he was not chlorinating which meant he exceeded the 1000 FC CFU/100 ml limit. The sample which was collected approximately three feet from the stern of his boat had 12,000 fecal coliform CFU/100 ml and 22,000 E. coli CFU/100 ml. This person was brought before the Wareham Board of Health for a hearing.

Only 10 samples were collected on this date and four were within the marina. The bacterial counts upstream of the marina were already elevated above shellfish standards and would have masked a problem at the marina except for the boater who discharged his toilet. Also, river flow and tidal exchange would also make it difficult to determine how much fecal waste was contributed to the river from the marina. An example was station 0906 - sampled behind the boat that was discharged. As stated, the fecal coliform count was 12,000 CFU/100 ml. The sample from station 0907 was collected at the same location as 0906 after a few minutes had passed. The fecal coliform count at this time was 40 CFU/100 ml. This reduction resulted from two factors: dilution and current which washed away the discharged material which held together as a unit before breaking up. No significant correlations were found between bacteria and chemical and physical parameters on this date.

On August 10 two marinas without pump-out facilities were sampled (i.e., Pt. Independence Yacht Club and Onset Bay Marina). The fecal coliform counts on August 10 were relatively low. No discharger was noted on this date although behind one boat the count was 330 CFU/100 ml. Although no conclusive results were found at these marinas, an indication of bacterial problems emanating from marinas can be noted by the following trend. All samples collected outside of the marina had lower fecal coliform values than the means of those collected within the slipways. These counts were 20,5, and 7.5 CFU/100 ml outside the marinas and a mean of 48 CFU/100 ml at Point Independence and 72 CFU/100 ml at Onset Bay marina.

The results of water quality sampling at Onset Bay were similar to those of Faust (1982) who sampled in Chesapeake Bay over a Labor Day weekend and found elevated bacterial densities related to the number of boats and boaters in a confined area. Her sampling was conducted in the morning on an outgoing tide which was similar to this sampling design. There were approximately 40 boats in the area. The fecal coliform density before the arrival of the boats averaged 3/100 ml and increased to 28/100 ml with increased boat activity.

9.0 LABORATORY RESULTS

SOURCE DIFFERENTIATION

During this first year of investigation problems in the area of sample collection and in the interpretation of laboratory results were encountered and severely affected the source differentiation efforts. In addition, the dearth of published material on the subject of source differentiation made it difficult for comparative interpretation to be made. The U.S. Environmental Protection Agency has not conducted studies on source differentiation on environmental samples. It has done extensive work on the development of laboratory techniques.

The pros and cons of using fecal streptococci to differentiate sources of fecal contamination are presented in Table 13. As a result of this study it appears that, regardless of time and money, it is critical that a high percentage if not all of the fecal streptococci colonies be biochemically differentiated. Only with statistically significant numbers can an investigator state conclusively that a known percentage of the fecal streptococci at a particular location was from livestock, birds, etc. This would aid in interpretation since the investigator could then do further sampling to determine the extent of impact by a particular source.

Bacteriological Indicators Employed in Study

In addition to the differentiation of fecal streptococci, several bacteriological testing procedures were employed to determine the concentrations of fecal coliform (mFC), E. coli (mTEC), enterococci (ME), Clostridium perfringens (mCP) and the most probable number of fecal coliforms using the relatively new A-1 test.

E. coli and enterococci have been suggested by EPA as standard setting bacteriological parameters for freshwater and marine bathing beaches, respectively. Cabelli in 1983 and Dufour in 1984 found these indicators to be more highly correlated with human enteric disease caused by the ingestion of fecally contaminated water while swimming than other bacteriological indicators included in their studies. Whether either of these indicators would highly correlate with enteric disease caused by the consumption of shellfish harvested from fecally contaminated waters can only be ascertained through an epidemiological study. This study by TSB gathered information on the use of different bacteriological media and test procedures and on the relative abundance of the various indicators in areas impacted by point and nonpoint sources of fecal contamination.

The Massachusetts Division of Water Pollution Control uses the mFC membrane filter test for fecal coliform bacteria enumeration for the classification of inland waters as shown in Table 4. The mFC test not only enumerates the fecally specific Escherichia coli present but also allows the development of other coliform bacteria that are able to carry out their metabolic functions under the conditions of the test, namely selective media environment and elevated temperature.

One advantage of using E. coli as a standard is that it is a fecally specific indicator, thus embodying a greater sanitary significance than other more

TABLE 13

USE OF FECAL STREPTOCOCCI TO DIFFERENTIATE SOURCES OF FECAL CONTAMINATION: PROS AND CONS.

PROs

- US EPA approved method which is described in Microbiological Methods, 1977.
- Way of determining if fecal streptococci from a particular type of source is impacting an area.

CONs

- Laboratory

    Difficult to interpret certain biochemical tests, especially starch hydrolysis, yet if it is incorrectly interpreted the fecal streptococci will be put into an incorrect group.

    Biochemical tests are very time consuming, labor intensive and expensive to run. LES estimates the cost per sample at approximately $110.

    Difficult to schedule source differentiation into a normal work week; some tests are seven days or longer.

    High false positives with KF media.

    S. bovis, *S. equinus* were not recovered from an area where they are likely to be present. This is probably because of their rapid die-off in the environment.

- Field

    Recovery of fecal streptococci is often low or non-existent in environmental samples.

    Difficult to interpret findings in the field with laboratory results. Much more work needs to be done in the examination of fecal contributions from nonpoint sources.

Recommendation - Discontinue use of this method as a way of differentiating sources of fecal contamination.

inclusive parameters. Another is that the mTEC test is judged to yield higher recoveries than the mFC procedure. The mTEC test has a two hour resuscitation period at 35|C before being transferred into a 44.5|C water bath. It is theorized that during this resuscitation period the enzyme systems of weakened bacteria undergo repair and as a consequence can survive and grow under elevated temperature conditions. In the mFC test, on the other hand, the bacteria filtered from a sample are immediately subjected to the elevated temperature environment which is used to differentiate fecal coliforms from non-fecal species.

To determine whether the mTEC test for E. coli produced higher recoveries than the mFC test all the data from the 1986 Buzzards Bay microbiological surveys were pooled and analyzed in the following fashion. Simple tallies were made of the number of occasions that the fecal coliform count using the mFC procedure equaled (53), was greater (118) and less (93) than the E. coli counts generated by the mTEC test. The data on these comparisons are presented in Table 14.

In Table 15 a scheme for defining fresh, brackish and saline waters appears and Table 16 contains tallies based on these categories. In brackish waters the number of times that FC densities exceeded those of E. coli and when they were less essentially equal as seen in Table 16. In saline waters the FC count was equal to E. coli 18 times and exceeded the E. coli 16 times. Only six times were the E. coli counts higher. The low numbers of E. coli and FC colonies that were recovered from saline waters (means of 14.3/100 ml and 19.8/100 ml, respectively) may influence these results. Due to the low counts, sample variability may play a role in this comparison study.

Further laboratory analysis is required to conclusively establish if mTEC recoveries were higher than mFC. Since the mFC-fecal coliform test will also recover Klebsiella sp., if present, this possibility may have contributed to the 118 times when the fecal coliform count exceeded that of the E. coli. If it is assumed that when the values of mFC equaled mTEC that only E. coli was recovered and then it would follow that 118 times either recovery was better with mFC or Klebsiella sp., was present and when the E. coli counts were higher this may indicate that the resuscitation period did have an effect.

Any additional testing to compare these two media should also include testing for Klebsiella sp.

Graphical Comparison of mFC and mTEC Recoveries

In a 1961 study by McCarthy et al a graphical comparison was presented on log probability paper of the results obtained by a direct membrane filter procedure and an MPN procedure for the determination of fecal coliform densities in seawater samples (mFC vs. EC MPN test). The results obtained in the Buzzards Bay study were similarly compared. The MPN A-1 test was used as the standard against which the results from mTEC for E. coli and the mFC test for fecal coliform were compared.

Prior to the development of ratios for the parameters selected for evaluation each MPN result obtained from the A-1 test was corrected for its positive bias in accordance with the Thomas prediction (1955). The ratios were calculated arrayed in order of magnitude and plotted on probability paper. The median A-1

TABLE 14

FECAL COLIFORM (FEC) MEMBRANE FILTER TALLIES COMPARED TO *E. coli* MEMBRANE FILTER TALLIES AND THE MEMBRANE FILTER TALLIES COMPARED TO FECAL COLIFORM MULTIPLE TUBE TEST (FECMPN)

FEC = *E. coli*	FEC > *E. coli*	FEC < *E. coli*
53	118	93
FEC = FECMPN	FEC > FECMPN	FEC < FECMPN
2	73	120
E. coli = FECMPN	*E. coli* > FECMPN	*E. coli* < FECMPN
0	71	122

TABLE 15

SCHEME FOR DEFINING FRESH, BRACKISH AND SALINE WATERS*

	SPECIFIC CONDUCTANCE µmhos/cm	CHLORIDE mg/l
Fresh	0 - 500	10 - 270
Brackish	>500 - 15,000	>270 - 13,500
Saline	15,000 - 60,000	>13,500 - 17,000

---

* Adopted in part from Cowardin et al (1979)

TABLE 16

E. coli MEMBRANE FILTER TALLIES COMPARED TO FECAL COLIFORM (FEC) MEMBRANE FILTER TALLIES UNDER FRESH, BRACKISH AND SALINE CONDITIONS

FRESH WATER

FEC = *E.coli*	FEC > *E. coli*	FEC < *E. coli*
1	1	3

BRACKISH WATER

FEC = *E. coli*	FEC > *E. coli*	FEC < *E. coli*
4	22	21

SALINE WATER

FEC = *E. coli*	FEC > *E. coli*	FEC < *E. coli*
18	16	6

MPN/mFC ratio was 1.12 (Figure 11), and the MPN/mTEC ratio was 1.16 for 94 tests that gave determinant values which could be used when the MPN is used as the standard for the purpose of comparing recoveries (even though it is estimated on a wider family, namely, fecal coliforms). As would be expected the fecal coliform test, mFC, yield higher bacterial counts than the E. coli, mTEC procedure. However, the proximity of the ratios to one indicates that the recovery by the membrane filter procedures were in general measuring the same bacteriological parameter, namely E. coli.

The same comparisons were made for the saline water samples. As a result of these statistical analyses the median ratios of MPN/mFC was 0.89 (Figure 13) and MPN/mTEC was 0.82 (Figure 14). This result was somewhat unexpected since these ratios indicate that the membrane filter procedures yielded higher counts than the A-1 MPN test. It has been well documented within the Shellfish Area Classification Program that the membrane filter (mFC) procedure for fecal coliform analysis is not an accepted procedure. In a 1955 study at the Lawrence Experiment Station it was found that the FC membrane filter procedure recovered only 10% of the FC population in a sample recoverable by the EC MPN procedure. One conclusion that can be drawn from these two studies is that the A-1 MPN procedure yields significantly lower recoveries of fecal coliform organisms in the samples included in this study. Theoretically, the reason for this difference may be associated with the media used in the two test procedures and the incubation temperatures used during the incubation period. Briefly, the A-1 employs an elevated temperature throughout the test period thus exposing attenuated and strong bacteria to this stress condition. The EC MPN fecal coliform procedure, on the other hand, employs a temperature and medium sequence, namely, a non-selective environment of tryptose broth for 24 hours at 35|C followed by a 48 hour incubation in selective EC broth at an elevated temperature. The harshness of the two procedures can be seen to favor the EC test for recoverability.

The E. coli (mTEC) test had only slightly better recoveries than the fecal coliform (mFC) membrane filter procedure when challenged by saline waters. It is not known if this increase in numbers relatively speaking is due to the two hour resuscitation period associated with the mTEC procedure. With the mFC test procedure the bacteria are immediately subjected to the elevated incubation temperature. The result of this comparison is at odds with the tallies presented in Table 16; however, the tallies revolved on a yes-no basis. The magnitude of the difference between the results obtained by the two tests was not considered in preparing these tables. In the graphical presentation of these ratios, the cumulative totals of the E. coli counts were slightly higher than the fecal coliform counts. However, the number of times when ratios were less than or equal to unity was the same for both the fecal and E. coli comparisons. In saline waters in 30 out of 42 samples analyzed, the MPN/FC or MPN/E. coli ratios were less than or equal to unity and therefore it follows that the membrane filter procedures yield higher estimates of fecal contamination in the samples analyzed than the A-1 MPN procedure.

Performance of Indicator Tests

A comparison of the counts obtained using the mFC and mTEC procedures for fecal coliform and E. coli, respectively, and a critique of the two test media by the Bacteriological Laboratory personnel of the Lawrence Experiment Station indicate that neither procedure is substantially more productive than the other. Both exhibited good colony formation and surpressed undesirable

FIGURE 11

Comparison of Fecal Coliform MPN (FEC MPN) Against Fecal Coliform Membrane Filter Test (mFC)

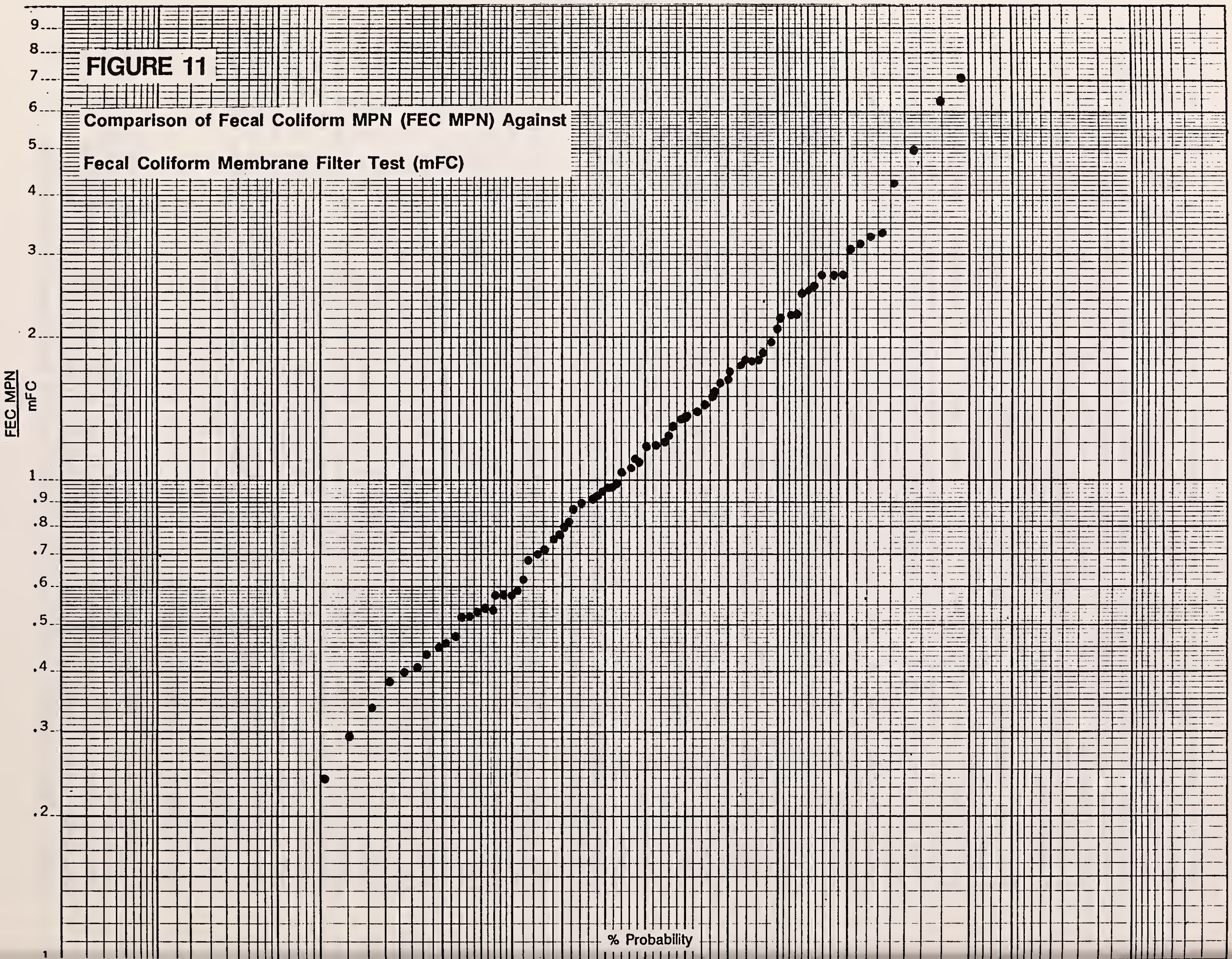
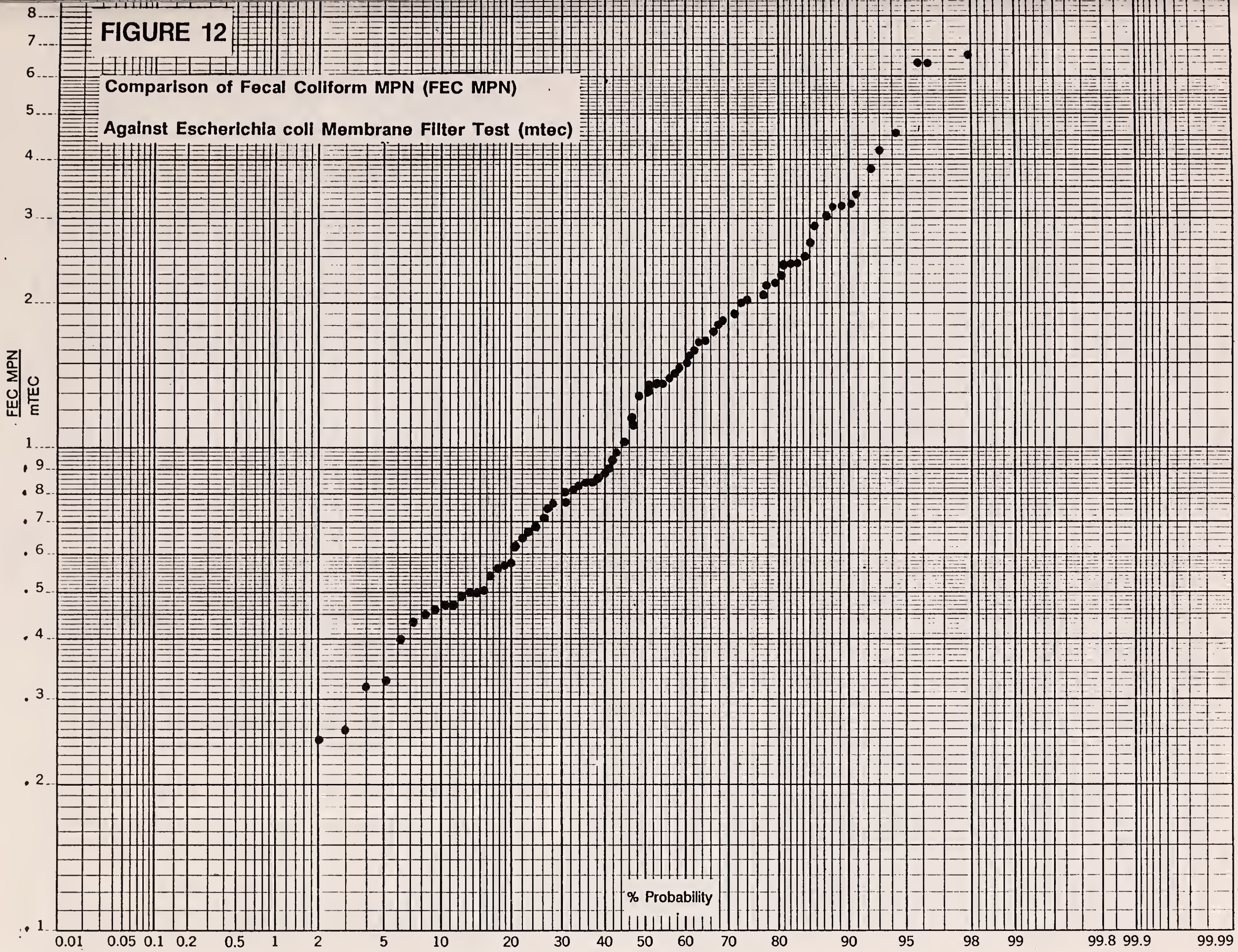


FIGURE 12

Comparison of Fecal Coliform MPN (FEC MPN)

Against Escherichia coli Membrane Filter Test (mtec)



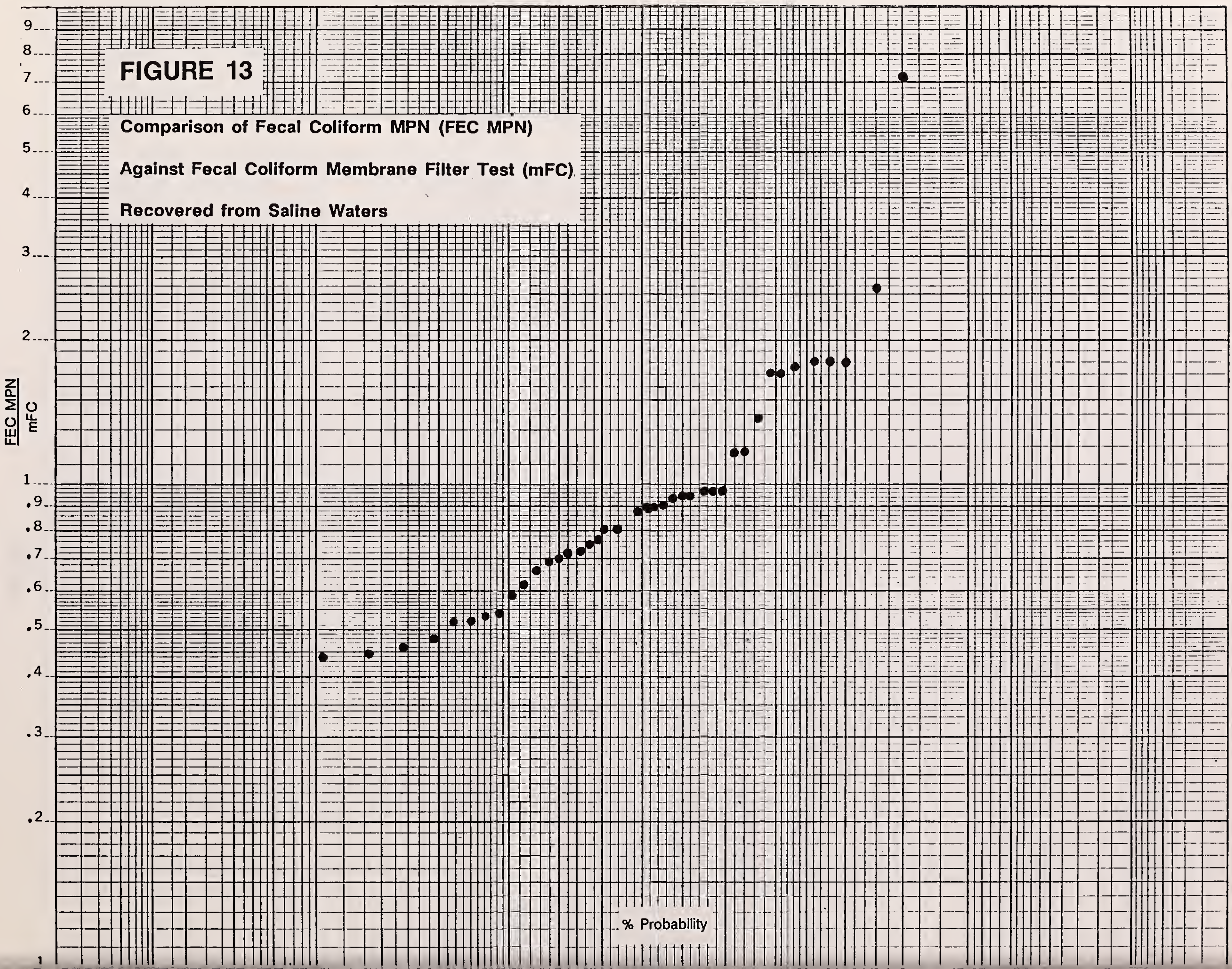


FIGURE 13

Comparison of Fecal Coliform MPN (FEC MPN) Against Fecal Coliform Membrane Filter Test (mFC) Recovered from Saline Waters

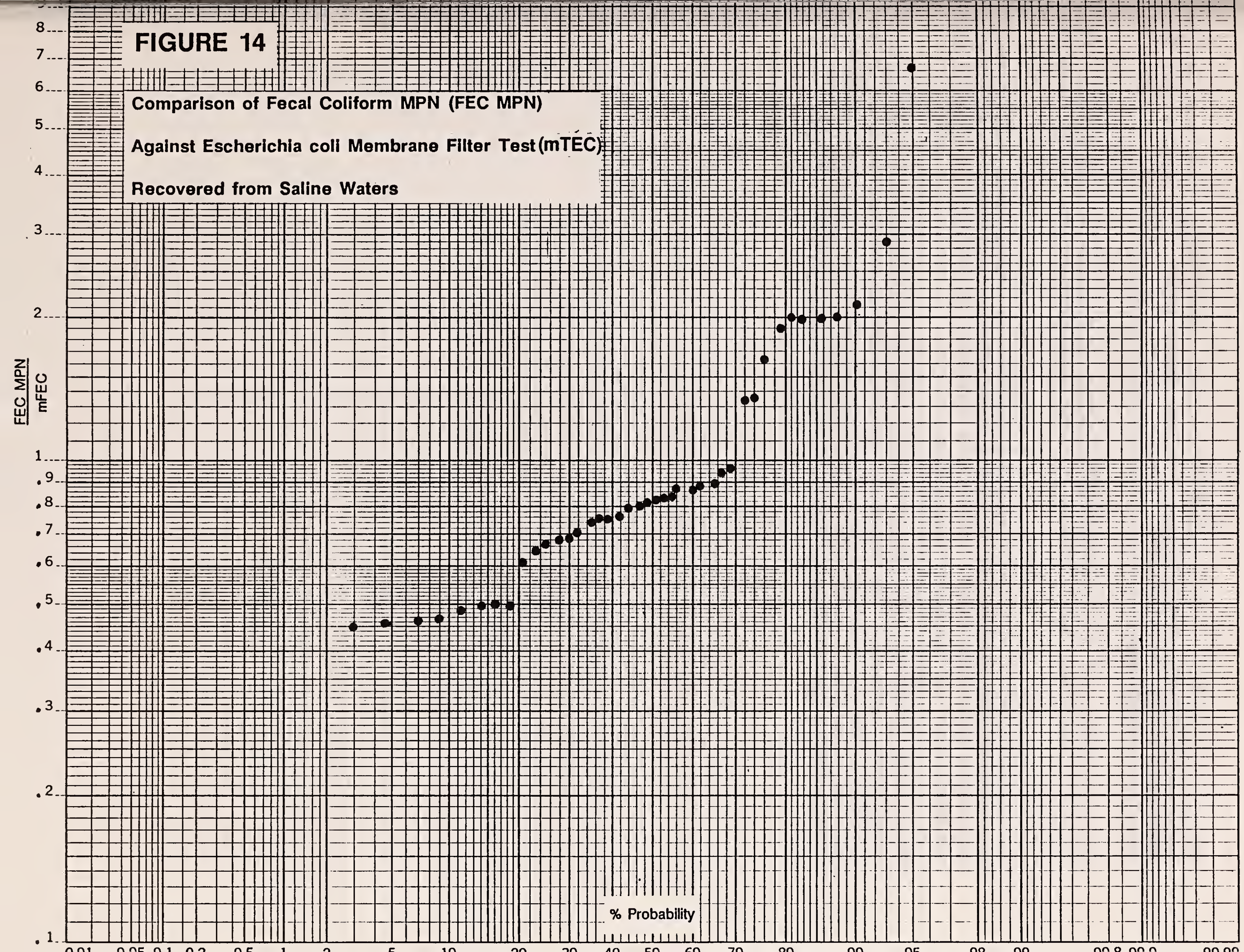


FIGURE 14

Comparison of Fecal Coliform MPN (FEC MPN)
Against Escherichia coli Membrane Filter Test (mTEC)
Recovered from Saline Waters

background growth. However, in areas with potentially high non-fecally specific Klebsiella populations, such as below paper mill discharges, the advantages of the mTEC procedure, namely, selectivity for E. coli, would be an important consideration.

The mCP medium and procedure for Clostridium perfringens was used only a few times in this study, but the media was easy to prepare and the colony formation was good.

The colony formation using the modified mE test for enterococci (Dufour, 1984) was not always as described for characteristic enterococci colonies (a red dot surrounded by a blue halo). On several occasions halo formation occurred but the development of the red circle did not.

There were problems with the KF medium used for isolation of the Group D streptococci bacteria. The colonies isolated with this media were used in the biochemical tests to differentiate the kinds of streptococci present. However, the false positive rate was often very high and on two occasions (7/9/86 and 8/26/86) there were 100 percent false positives (Table 17).

TABLE 17

RESULTS FROM TESTING OF COLONIES RECOVERED ON KF MEDIUM

DATE	#COLONIES TESTED	#CONFIRMED FECAL STREPTOCOCCI	#FALSE POSITIVES	% FALSE POSITIVES
6/10/86	6	6	0	0
7/9/86	23	0	23	100
7/21/86	58	32	26	44.8
8/11/86	56	45	11	19.6
8/26/86	17	0	17	100

10.0 CONCLUSIONS AND RECOMMENDATIONS

Water quality in Buzzards Bay was impacted in 1986 by nonpoint sources of fecal contamination resulting in closure of shellfish areas in both the Wareham and Westport Rivers. Although an effort was made to use various bacterial indicators to determine the actual types of sources impacting an area, not all sources were identified. Bacterial problems originating from a marina in Wareham and from pastured cows at Westport were confirmed.

Source differentiation of the fecal streptococci was a useful method but, during this first year of use, sample design limited the interpretation of the data. More colonies need to be chemically differentiated per plate. Also, with KF media there were problems with high false positives and S. bovis, an indicator of livestock, was never isolated although in the sample areas, cows were much in evidence. In these cases examination of the other fecal indicators helped in the interpretation of the kind of source. Another problem in relying upon source differentiation is that, for some warmblooded sources, fecal coliforms are present in much higher numbers than the fecal streptococci; when fecal coliform densities are reduced as a result of dilution, etc., fecal streptococci may not be isolated at all.

It was difficult to sample nonpoint sources for their contribution of fecal contamination. Tides, soils, and precipitation all influence contributions from nonpoint sources.

Only if a septic system has failed and is flowing overland will offshore sampling result in locating a source of fecal contamination to an area. The low numbers of fecal bacteria from leaching fields which may survive travel through soil or sediments can not be located because of the precision of the bacterial counts as well as background contributions.

Within shellfish areas, such as in the lower Wareham River, Muddy Cove, and the Westport River, fecal streptococci from birds were identified through the use of source differentiation as a significant contributor of fecal contamination. Also their relative percentage contribution may vary with the seasons. During the summer, fewer birds are on the water, but instead are on nests on the shore. Perhaps this led to the low recovery of group Q streptococci in July from the Wareham River while the highest percentage was recovered September 17 from the Westport River.

Educational programs established by citizens' groups or town agencies can be helpful in discouraging people from feeding geese, gulls, or ducks. The feeding of waterfowl can encourage them to alter their behavior and this has resulted in their attempting to overwinter here rather than flying south.

Additional bacterial work should be done at the Wareham River estuary, Wareham and the Wareham and Agawam Rivers. Bacterial problems have been documented at Warr's Marina, but additional work is needed to investigate storm drains and other pipes for possible illegal tie-ins because of the elevated bacterial counts found in the estuary.

Marsh areas appear to be a problem as both a potential source of fecal coliform bacteria, as well as providing an excellent habitat for multiplication of the fecal coliform bacteria. There was some evidence of increased fecal coliform densities occurring within the Muddy Cove salt marsh. There was also evidence of possible boat wastes within one channel.

In contrast, two other areas sampled with extensive marshland (Sunset Cove and Broad Marsh Cove) had very low fecal coliform counts when sampled. It is believed that this is because of onshore winds which held the "cleaner" ocean water over the marsh and acted to dilute any bacterial counts. However, other attributes may also be significant, such as, the amount of fresh water input, circulation patterns, etc. and these need to be examined further.

Fecal problems were indicated at the three marina areas sampled in Wareham. These resulted from the lack of pump-out facilities or the lack of use of the pump-out facilities that were provided. At the marinas at Onset Bay without pump-out facilities (Point Independence Yacht Club; Onset Bay Marina) slightly elevated bacterial counts were found within the slipways. This may indicate that people are discharging their toilets into the marina areas. At Warr's Marina a boat owner discharged his toilet even though a pump-out facility is provided.

It is recommended that some plan be devised to encourage the construction of pump-out facilities at marinas. Also, a system should be established at each marina to track which boats are being pumped out and when. An effort will be made by DWPC/TSB personnel to work with those marina operators who wish assistance in formulating a plan.

There was not a reasonable pattern established for the regression lines derived from log-transformed enterococci to log-transformed streptococci data. In part, this may result from the somewhat contrived situation created by the use of a portion of the fecal streptococci colonies. Patterns for types of sources may be evident when the source differentiation database is increased.

In a graphical comparison of data collected from fresh, brackish and marine waters, the fecal coliform recovered with mFC had a slightly higher recovery than the E. coli (mTEC), but the fecal coliform MPN recoveries were slightly higher than both. In saline waters, the membrane filter tests (mFC, mTEC) exceeded those of the MPN; the E. coli (mTEC) had a slightly better recovery than the fecal coliform (mFC).

The mTEC medium for the isolation of E. coli was found by LES to have good colony formation and low numbers of false positives. The mCP for Clostridium perfringens was found to have good colony formation and little background growth while the enterococci colonies isolated by the mE medium did not always have the typical colony characteristics. The fecal streptococci colonies isolated with KF medium had good colony formation, but high percentages of false positives were found.

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SAMPLING DESIGN*

Samples were typically collected on Mondays, beginning on June 17, 1986 and ending on October 8, 1986. In most cases, samples were collected on a falling tide. An effort was made to sample during the worst-case conditions for each particular nonpoint source. The nonpoint sources chosen for examination were:

1) A marina with a pump-out facility for septage;

2) A marina without a pump-out facility for septage;

3) Houses in compliance with Title V;

4) Houses not in compliance with Title V;

5) Salt marsh areas;

6) Agricultural areas;

7) Bird populations on the water; and

8) Storm drains/parking lot runoff.

The marinas were to be sampled on busy, hot weekend mornings when boating use was heavy. However, the summer of 1986 was very wet with 13 weekends exhibiting some precipitation (National Oceanic and Atmospheric Administration, 1986). Several sampling attempts had to be cancelled. The marinas were finally sampled with some boaters present, although on August 3 rain was threatening and on August 10, the second time that the marinas were sampled, rain was forecast. The marinas chosen for study were: Warr's Marina on the Wareham River, which has a septage pump-out facility for boats, and the Point Independence Yacht Club and the Onset Bay Marina, both of which are on Onset Bay, Wareham. Neither of these has pump-out facilities.

Title V is a state regulation which includes setback requirements for cesspools and septic tank leaching fields from surface waters. Currently, 50 feet is required. In order to examine if this distance is adequate for the removal of bacteria in areas with sandy soils such as Buzzards Bay, two types of housing areas were chosen for study. These areas are located on the East Branch of the Westport River. The west side of the East Branch of the Westport River below Hix Bridge has an area of houses which are apparently in compliance with Title V. Another area on the west side has a section with homes that are apparently not in compliance with Title V. These distinctions were made by observation and not by measuring the actual distances between the leaching fields and the water's edge. Sampling was done on the ebb part of a spring tide which was believed to represent the worst-case conditions.

Locations with agricultural impacts were chosen for study along the East Branch of the Westport River above Hix Bridge. Agricultural inputs were chiefly from grazing cattle, but some corn fields were also present in the study areas. Samples were collected along the shore and in drainage channels emanating from cow pastures. There are 32 active dairy and beef cattle farms along the East Branch of the Westport River, which have a total cow population of 3,900 (Westport River Watershed Committee, 1983).

Other nonpoint sources of fecal contamination were studied in Wareham in the Agawam, Wareham and East River basins and in Onset, Sunset and Muddy coves. Sampling locations were chosen to examine fecal contributions from the Wareham Publicly Owned Treatment Works (POTW), salt marsh areas, marinas, bird populations, and storm drains. However, during the sampling period migrating birds did not flock in the coves and marshes, so they were not sampled. Because no suitable rain storms occurred during the sampling weeks, storm drains were not sampled either.

In the Agawam River, sampling was conducted above and below the Wareham POTW. Bacterial sampling included analysis of Clostridium perfringens, as well as fecal coliform, enterococci and fecal streptococci bacteria. Triplicate samples were collected at each location in the river. The effluent from the Wareham POTW passes through sand filters before it is discharged into the Agawam River. There are four separate discharge pipes at the Wareham POTW. If the Clostridium counts had been elevated as well as the other bacterial parameters, then an effort would have been made to use the Clostridium perfringens as a tracer of the sewage treatment plant discharge. The bacterial counts, however, remained about the same at all sampling stations in the Agawam River chosen for study on that day. In addition, the Clostridium perfringens counts were low.

In the first few weeks of the study, samples were collected at 0', 50' and 100' intervals offshore. This sampling regime was abandoned when results showed no significant differences between these intervals. It had been hoped that a sample gradient would have been observed. Since a density gradient was never observed the sampling regime was altered so that more shoreline areas could be sampled.

In addition to the shoreline sampling for several indicator organisms, samples were also collected for differentiation of the fecal streptococci bacteria into their probable sources (U.S. EPA, 1978). Those included in the differentiation were: insects, warmblooded animals and birds, and vegetative sources. This method was chosen because of its potential for use in managing areas impacted by nonpoint sources of pollution.

The sampling schedule for the Agawam and Wareham River basins was designed so that upper basin areas were sampled first for their bacterial assemblage and then each following week another nonpoint source further downstream as chosen for examination. Finally, samples were collected for source differentiation of the fecal streptococci bacteria in an area of the Wareham River offshore and below the Warr's Marina.

Source differentiation was also conducted at the East Branch of the Westport River upstream of the shellfish closure line which presently is located just below Great Island. Source differentiation was also accomplished at Muddy Cove in Wareham. A similar effort was made to do the same at Sunset Cove, Wareham, but on two separate occasions fecal streptococci colonies were not isolated from samples obtained from this area.

* References Cited

National Oceanic and Atmospheric Administration. 1986. Record of River and Climatological Observations for New Bedford and Acushnet, MA. (Unpublished memo).

Westport River Watershed Committee. 1983. Rural Clean Water Program Westport River Watershed Project. Westport River Rural Clean Water Program. p108.

US Environmental Protection Agency. 1978. Microbiological Methods for Monitoring the Environment Water and Wastes. Cincinnati, Ohio. pp135-153.

FIELD METHODS AND MATERIALS*

Samples were collected from a Boston Whaler. Bacterial samples were collected in sterile glass containers held below the surface of the water (American Public Health Assoc., 1985). Samples were also collected for chlorides, specific conductivity, hardness, and suspended solids in a half-liter glass bottles. This bottle was previously rinsed twice with sample water and then filled to the shoulder of the bottle. If the water depth was more than four feet, a Kemmerer vertical water sampler was used to collect the sample. For sample depths less than four feet a surface grab sample was typically collected. Nutrient samples were also collected in half-liter bottles which were rinsed with sample water twice. Samples were analyzed for total phosphorus, Kjeldahl-nitrogen and ammonia-nitrogen. The nutrient samples were fixed in the field with 2 mls of concentrated sulfuric acid. A temperature compensated Orion meter as used for pH and temperature measurements in the field. When available, a Y.S.I model 33 salinity meter was used for specific conductivity and salinity.

All samples were placed in coolers containing ice and brought to the Lawrence Experiment Station for analysis. All samples were brought to LES within the six-hour holding period for the bacterial samples.

* Reference Cited

American Public Health Assoc. 1985. Standard Methods for the Examination of Water and Wastewater. 16th Edition. American Public Health Assoc., New York. p1268.

LABORATORY MATERIALS AND METHODS

Membrane Filtration

Depending upon the source of water, different volumes of water samples were passed through presterilized membrane filers (HA type; Millipore, 0.45 um pore size). If the sample volume was insufficient to provide an even distribution of bacteria on the filters then phosphate buffered $MgCl_2$ solution was first added to the funnels and the sample was added in turn to ensure accurate colony counts. Filter holders and funnels were sterilized for two minutes in an ultraviolet sterilizing apparatus.

Recovery Media

The following Difco media were prepared and used according to the manufacturer's instruction and those included in Standard Methods for the Examination of Water and Wastewater (American Public Health Assoc., 1985): KF, mFC, MTEC, A-1. The mE media was prepared and used according to the method of M.A. Levin (Levin et al 1975) as modified by A.P. Dufour (Dufour, 1980). The mCP media was prepared and used according to the method of Bisson and Cabelli (1979). A-1 (Difco) was prepared and used according to the manufacturer's instructions and the 12 tube MPN chart developed by James H. Redman (1974). The mTECH was prepared and used according to the manufacturer's instructions and Dufour et al., 1981.

The A-1 was used as an MPN test. The KF, mCP, mE, mFC, and mTECH were used in membrane filter procedures.

Incubation Periods

Inoculated A-1 tubes were incubated for a two-hour resuscitation period at 35|C (Lab Line-Environette) and then transferred to a 44.5|C water bath incubator (Blue M or GCA/Precision scientific) for 24 hours. The KF plates were incubated for 48 hours at 35|C (Lab-Line Environette). The mCP plates were incubated in a BBL Gas Pack Anaerobic jar for 24 hours at 45|C (Forma-HydroJac incubator). The mE plates were incubated at 41|C for 48 hours (Blue M). The mFC plates were sealed in watertight plastic bags (Whirlpac) and incubated in a 44.5|C water bath incubator (Blue M or GCA precision scientific) for 24 hours. The mTECH plates were incubated for a two-hour resuscitation period at 35|C (Lab-Line Environette) and then sealed in watertight plastic bags (Whirlpac) and transferred to a 44.5|C water bath incubator for 24 hours.

Source Differentiation of Fecal Streptococci

If a sampling area was so designated, a representative amount of characteristic colonies growing on KF media were picked and streaked onto BHI agar slants (Difco) for source differentiation. Colonies were differentiated according to their sources, i.e., warmblooded animal, bird, insect, and vegetation. The methods used for differentiation were according to Microbial Methods for Monitoring the Environment (1978) and included the following tests: growth at 45|C, growth at 10|C, catalase production, starch hydrolyzes, lactose fermentation, growth in 6.5% NaCl BHI broth, growth in pH 9.6 BHI broth, reduction of 0.1% methylene blue in milk and peptonization of litmus milk.

Quality Control

For purposes of quality control duplicate samples were collected and analyzed on a predetermined schedule. Typically every other week duplicates were analyzed for all tests except the A-1 MPN test.

Chemical and Physical Parameters

Samples for chemical and physical analysis were brought to the Lawrence Experiment Station (LES). Table A-1 is a list of analytical methods used at LES.

* References Cited

American Public Health Assoc. 1985. Standard Methods for the Examination of Water and Wastewater. 16th Edition. American Public Health Assoc., New York. p1268.

Bisson, J.W. and V.J. Cabelli. 1979. "Membrane Filter Enumeration Method for *Clostridium perfringens*. *Applied and Environmental Microbiology*. 37:55-66.

Dufour, A.P. 1980. *Abstracts of the Annual Meeting, American Society for Microbiology*. Abstract #Q69. American Society for Microbiology, Washington, D.C.

Dufour, A.P., E.R. Strictland and V.J. Cabelli. 1981. "Membrane Filter Method for Enumerating *Escherichia coli*." *Applied and Environmental Microbiology*. 41: 1152-1158.

Levin, M.A., J.R. Fisher and V.J. Cabelli. 1975. "Membrane Filter Technique for the Enumeration of Enterococci in Marine Waters." *Appl. Microbiology*. 30:66-71.

Redman, J.H. 1974. "A Simpler Multiple Fermentation Tube Test for Monitoring the Bacteriological Quality of Shellfish Harvest Waters. Quality of Shellfish Harvest Waters. The Examination of Twelve 1.0 in Sample Portions." IN: *Proceedings of the 8th National Shellfish Sanitation Workshop*. Edit. D. Wilt. U.S. Public Health Service, New Orleans. pp123-131.

TABLE A-1

ANALYTICAL METHODS USED AT LAWRENCE EXPERIMENT STATION*

PARAMETER	METHOD	REPORTED AS
Suspended Solids	Filtration through standard glass fiber filter paper. Residue dried at 103-105|C. Gravimetric	mg/l S.S.
Total Kjeldahl-Nitrogen	Acid digestion using Technical BD-40 Block Digester. Colorimetric analysis (reaction of ammonia, sodium salicylate, sodium nitroprusside, and sodium hypochlorite in buffered alkaline medium) using Technicon Auto Analyzer II	mg/l TKN
Ammonia-Nitrogen	Phenate method, automated. Colorimetric analysis using Technicon Auto Analyzer II	mg/l NH_3-N
Nitrate-Nitrogen	Hydrazine reduction method, automated. BD-40 Block Digester. Ascorbic acid reduction colorimetric method using Technicon Auto Analyzer II	mg/l P
Conductivity	Wheatstone Bridge type meter. Yellow Springs Instrument conductivity bridge, Model 31	µmhos/cm
Chloride	Argentometric (titration with silver nitrate)	mg/l Cl
Hardness (Ca+Mg)	Atomic Absorption Spectrophotometry. Air-acetylene flame. Perkin-Elmer Model 403	mg/l

TABLE A-2

MEAN VALUES OF PHYSICAL, CHEMICAL AND BACTERIAL PARAMETERS IN FRESH, BRACKISH, AND SALT WATER SAMPLES BUZZARDS BAY, 1986

	FRESH WATER	BRACKISH WATER	SALT WATER
Total Kjeldahl Nitrogen (mg/l)	1.4	2.0	1.6
Ammonia (mg/l)	0.4	0.1	0.1
Total Phosphorus (mg/l)	0.3	0.2	0.1
Dissolved Oxygen (mg/l)	--	6.9	6.9
pH (standard units)	6.7	7.1	7.7
Fecal Coliform Bacteria CFU/100 ml)	19600	2560	20
E. coli (CFU/100 ml)	26600	2476	14
Enterococci Bacteria (CFU/100 ml)	6400	202	7
Streptococci Bacteria (CFU/100 ml)	6400	369	7
Hardness (mg/l)	19	2615	4812
Suspended Solids (mg/l)	10.1	6.4	6.9

TABLE A-3

MEAN VALUES OF BACTERIAL INDICATORS MEASURED AT BUZZARDS BAY, 1986

DATE	STATIONS	FECAL COLIFORM BACTERIA CFU/100 ml	ENTEROCOCCI BACTERIA CFU/100 ml	FECAL STREPTOCOCCI BACTERIA CFU/100 ml	*Escherichia coli* BACTERIA CFU/100 ml
6/23	0201-0212	331	—	187	300
6/30	0301-0305	1065	714	847	684
7/7	0401-0412	8	5	8	8
7/9	0501-0510	43	3	13	32
7/14	0601-0610	2660	2342	2258	3295
7/21	0701-0712	153	24	95	133
7/28	0801-0812	54	3	3	65
8/3	0901-0910	1397	21	33	2507
8/4	1001-1013	4718	155	262	5115
8/10	1101-1115	49	12	8	9
8/11	1201-1212	188	58	268	176
8/19	1301-1312	457	45	69	284
8/26	1401-1409	1	8	8	1
9/17	1501-1510	756	67	569	607
9/24	1601-1610	3	2	2	4
10/2	1701-1712	14125	327	3625	15558
10/8	1801-1808	6	4	3	6
All Stations		1514	386	440	1673

TABLE A-4

MEAN VALUES OF THE PHYSICAL PARAMETERS MEASURED AT BUZZARDS BAY, 1986

DATE	STATIONS	DISSOLVED OXYGEN (mg/l)	pH (STANDARD UNITS)	SUSPENDED SOLIDS (mg/l)	SPECIFIC CONDUCTIVITY (umhos/cm)	TEMPERATURE (°C)
6/23	0201-0212	6.8	6.6	6.4	8425	21.0
6/30	0301-0305	8.1	5.3	4.8	—	21.5
7/7	0401-0412	6.6	7.4	7.5	—	24.0
7/9	0501-0510	7.8	7.8	8.9	27775	25.8
7/14	0601-0610	6.6	7.1	10	—	20.8
7/21	0701-0712	6.2	6.8	12.4	28750	24.8
7/28	0801-0812	8.8	7.1	12.9	27929	27.0
8/3	0901-0910	—	7.1	6.2	—	25.0
8/4	1001-1013	6.7	7.0	6.0	—	26.0
8/10	1101-1115	—	7.9	5.5	—	22.7
8/11	1201-1212	8.1	7.1	6.2	—	25.4
8/19	1301-1312	5.8	7.6	7.5	—	22.5
8/26	1401-1409	7.1	7.7	6.9	—	22.9
9/17	1501-1510	7.6	7.1	4.4	—	17.1
9/24	1601-1610	6.2	7.4	8.7	—	18.9
10/2	1701-1712	—	6.8	8.3	—	17.7
10/8	1801-1808	8.0	8.0	15.7	—	13.9
All Stations		7.1	7.2	7.9	23355	20.9

TABLE A-5

MEAN VALUES OF CHEMICAL PARAMETERS MEASURED AT BUZZARDS BAY, 1986

DATE	STATIONS	TOTAL KJELDAHL NITROGEN (mg/l)	AMMONIA NITROGEN (mg/l)	TOTAL PHOSPHORUS (mg/l)	TOTAL HARDNESS (mg/l)	CHLORIDE (mg/l)
6/23	0201-0212	2.08	0.31	0.16	1010	--
6/30	0301-0305	1.12	0.19	0.16	614	--
7/7	0401-0412	1.41	0.26	0.11	3142	--
7/9	0501-0510	2.40	0.19	0.13	2461	--
7/14	0601-0610	2.21	0.17	0.18	1752	--
7/21	0701-0712	2.00	0.08	0.15	3994	--
7/28	0801-0812	1.51	0.06	0.14	3536	--
8/3	0901-0910	2.20	0.13	0.15	2836	11429
8/4	1001-1013	2.30	0.08	0.15	2401	9997
8/10	1101-1115	1.65	0.04	0.14	5585	16396
8/11	1201-1212	2.40	0.14	0.15	1334	6575
8/19	1301-1312	1.36	0.18	0.12	4180	13771
8/26	1401-1409	1.08	0.08	0.10	5457	15194
9/17	1501-1510	1.70	0.06	0.20	3751	9688
9/24	1601-1610	1.90	0.10	0.09	4030	16160
10/2	1701-1712	1.70	0.32	0.26	694	3729
10/8	1801-1808	1.56	0.05	0.06	3796	17192
All Stations		1.8	0.15	0.14	3115	11831

TABLE A-6

SUMMARY OF SOURCE DIFFERENTIATION PERCENTAGES

FROM BUZZARDS BAY, 1986

DATE	NON-FECAL STREPTOCOCCI	BIRDS	WARM-BLOODED ANIMALS	LIVESTOCK AND POULTRY	INSECTS	VEGETATION
7/21	45	2	48	0	5	0
8/11	17.6	15.7	35.3	0	31.4	0
9/17	2.1	22.9	75	0	0	0
10/2	6.6	1.7	90	0	1.7	0

TABLE A-7

SUMMARY OF BACTERIAL CORRELATIONS FROM SOURCE DIFFERENTIATION DATES

Log-Transformed Streptococci With Log-Transformed Fecal Coliform

DATE	r	r^2	SIGNIFICANCE $p<0.05$
7/21	0.4872	0.2373	NO
8/11	0.7261	0.5272	YES
9/17	0.7795	0.6076	YES
10/2	0.7666	0.5876	YES

Log-Transformed Enterococci With Log-Transformed Streptoccoci

DATE	r	r^2	SIGNIFICANCE $p<0.05$
7/21	0.7974	0.6359	YES
8/11	0.8787	0.7721	YES
9/17	0.7685	0.5906	YES
10/2	0.8328	0.6936	YES

TABLE A-8

STATION LOCATIONS - MUDDY AND BROAD COVES, WAREHAM

JUNE 17, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
0101	Off of salt marsh area, east side of Muddy Cove at approximately 20th Street, 50′ from shore	41°45′13"	70°39′27"
0102	Off of salt marsh area, east side of Muddy Cove at approximately 20th Street, 100′ from shore	41°45′13"	70°39′29"
0103	35′ from shore approximately 50′ downstream of 0101	41°45′03"	70°39′25"
0104	100′ from shore, approximately 50′ downstream of 0101	41°45′03"	70°39′29"
0105	Point of land on western shore approximately 60′ from shore, just above Dummy Bridge	41°44′54"	70°39′28"
0106	200′ downstream of 0105, 50′ from shore in shellfish area	41°44′54"	70°39′25"
0107	West of Dummy Bridge in center of channel	41°44′54"	70°39′18"
0108	North of town boat ramp, approximately 35′ from shore	41°44′49"	70°39′21"
0109	North of town boat ramp, approximately 120′ from shore	41°44′49"	70°39′17"
0110	Gibbs Brook, at Cranberry Greens Mini Golf (Route 6 - Cranberry Highway)	41°45′25"	70°39′13"

Figure A-1

STATION LOCATIONS

MUDDY and BROAD COVES

17 JUNE 1986

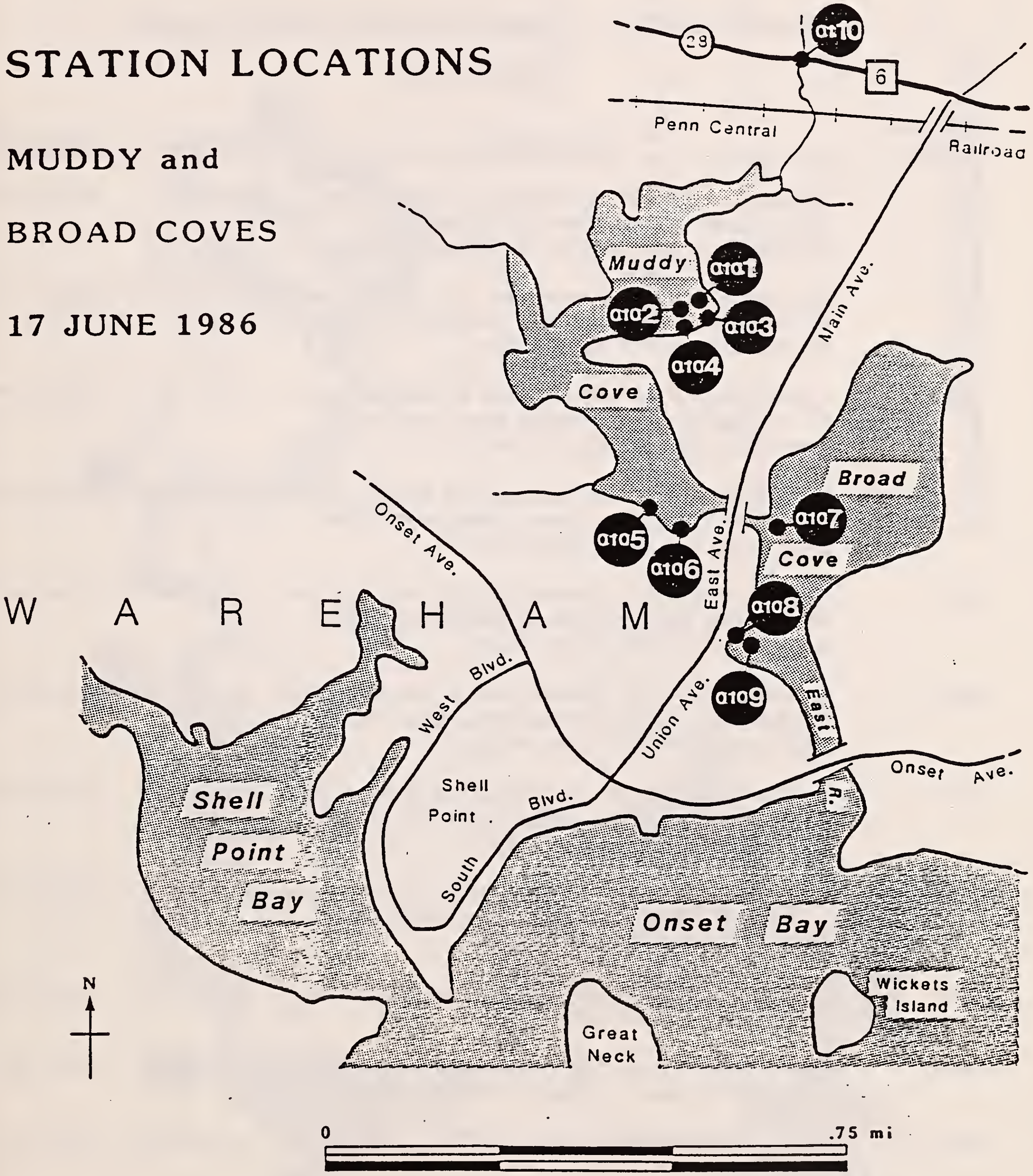

TABLE A-9

STATION LOCATIONS - AGAWAM AND WAREHAM RIVERS, WAREHAM

JUNE 23, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
0201	Approximately 400′ upstream of first discharge pipe at Wareham Publicly Owned Treatment Plant located on Agawam River	42°35′00"	70°40′40"
0202	Approximately 50′ downstream of first pipe at Wareham Publicly Owned Treatment Plant, approximately 50′ from shore	42°34′58"	70°40′41"
0203	Approximately 50′ downstream of second pipe at Wareham Publicly Owned Treatment Plant, approximately 50′ from shore	42°34′57"	70°40′42"
0204	Approximately 50′ downstream of second pipe, at Wareham Publicly Owned Treatment Plant, approximately 100′ from shore	42°34′56"	70°40′39"
0205	Approximately 50′ downstream of fourth pipe at Wareham Publicly-Owned Treatment Plant, approximately 20′ from shore	42°34′56"	70°40′43"
0206	Approximately 50′ downstream of fourth pipe at Wareham Publicly Owned Treatment Plant, approximately 100′ from shore	42° 34′55"	70°40′42"
0207	Approximately 300′ downstream of fourth pipe at Wareham Publicly Owned Treatment Plant, approximately 50′ from shore	42°34′55"	70°40′43"
0208	Approximately 300′ downstream of fourth pipe at Wareham Publicly Owned Treatment Plant, approximately 100′ from shore	42°34′54"	70°40′43"
0209	Just downstream of Sandwich Road (Route 6) Bridge, on Agawam River	41°46′25"	70°41′15"
0210	Under power lines just downstream of house on point of land close to Sandwich Road	41°45′40"	70°41′50"
0211	Off bird sanctuary, approximately 100′ from shore	41°45′34"	70°42′37"
0212	Beside channel marker 30 in Wareham River	41°44′54"	70°42′21"

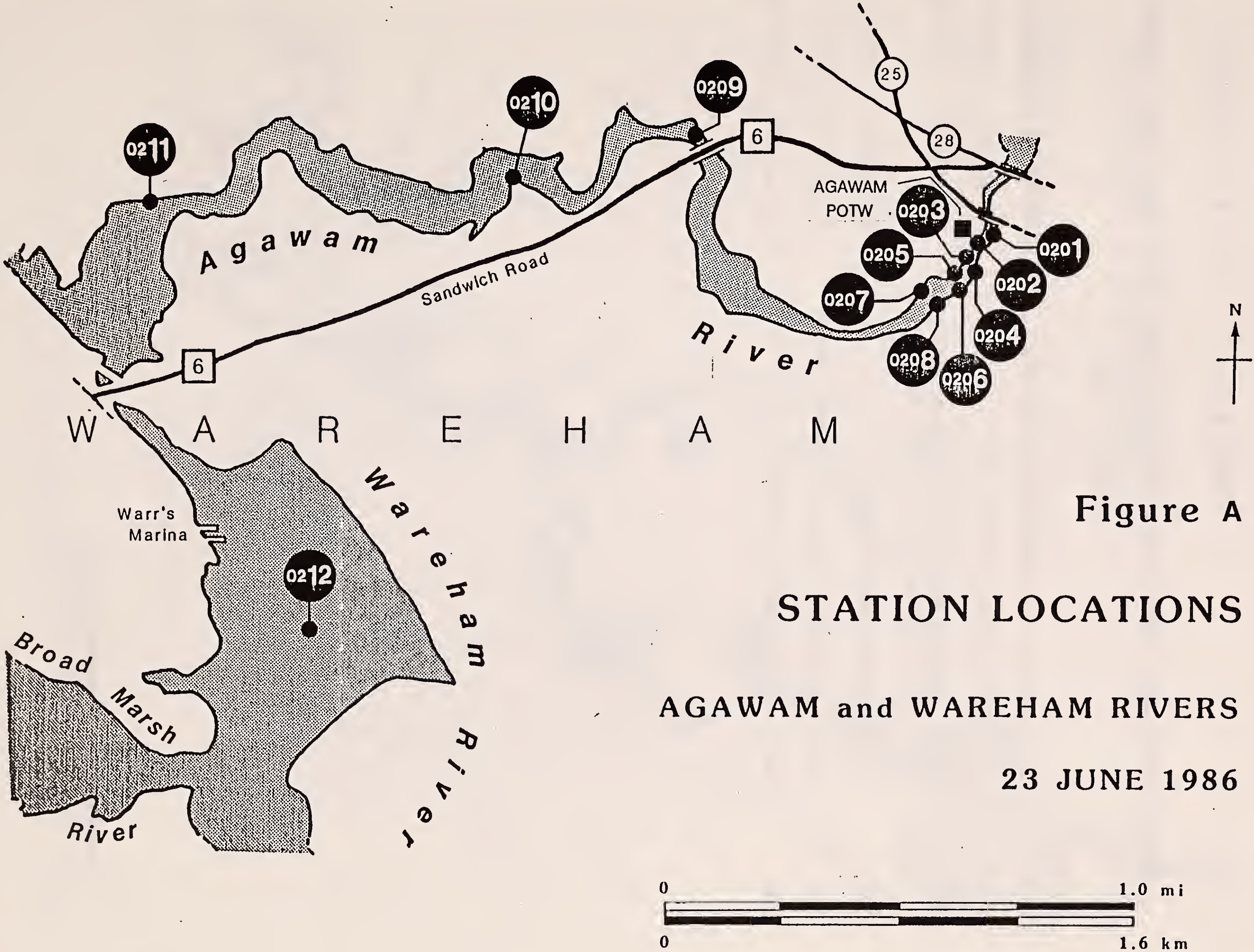

Figure A - 2

STATION LOCATIONS

AGAWAM and WAREHAM RIVERS

23 JUNE 1986

TABLE A-10

STATION LOCATIONS - UPPER AGAWAM RIVER AND SHELL POINT BEACH SITES, WAREHAM

JUNE 30, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
0301	Outlet Mill Pond at Route 28	41°45’45"	70°40’35"
0302	Maple Springs Brook off of Maple Springs Road near confluence with Mill Pond	41°46’45"	70°40’20"
0303	Outlet Glenn Charlie Pond	41°47’00"	70°39’25"
0304	Beach in Sunset Cove next to Town Beach	41°44’07"	70°43’27"
0305	Wareham Town Beach at Shell Point	41°44’06"	70°43’19"

Figure A-3

STATION LOCATIONS

UPPER AGAWAM RIVER

30 JUNE 1986

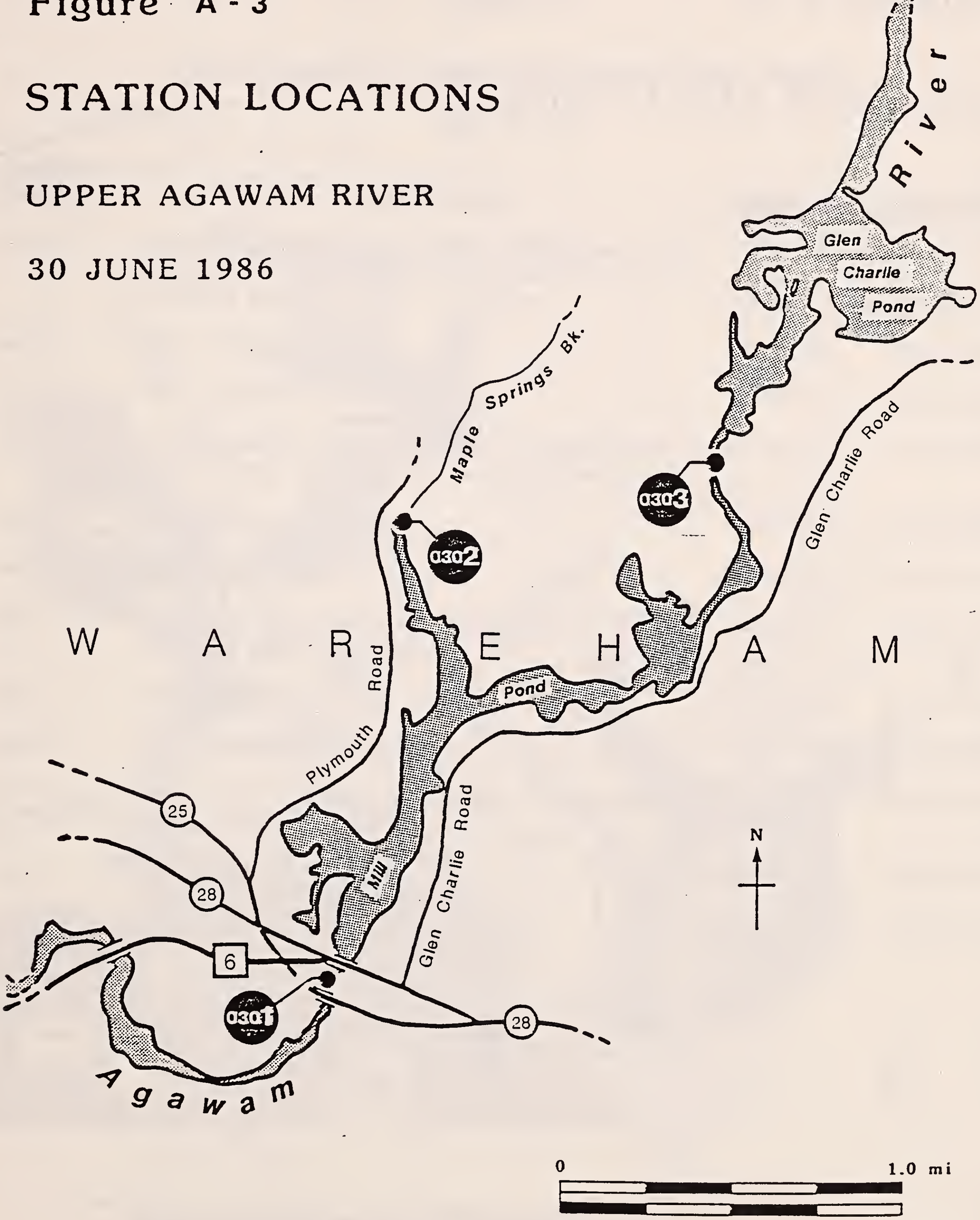

Figure A - 4

STATION LOCATIONS

SHELL POINT

30 JUNE 1986

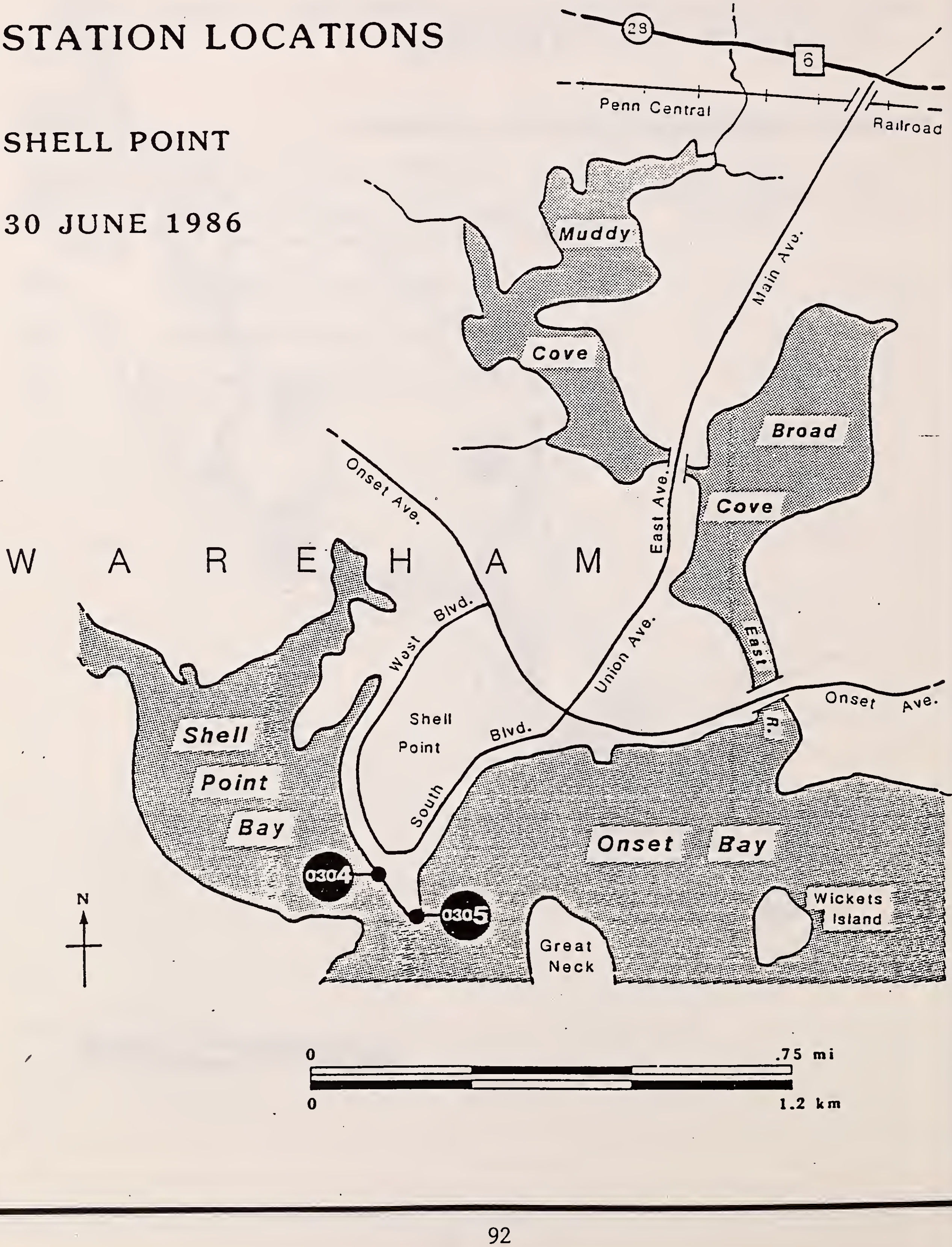

TABLE A-11

STATION LOCATIONS - AGAWAM AND WAREHAM RIVERS, WAREHAM

JULY 7, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
0401	Sampled along eastern shore downstream of buoy 28, sampled onshore in front of brown 2-story house	41°45′03"	70°42′09"
0402	50′ offshore of 0401	41°45′03"	70°42′12"
0403	100′ offshore of 0401	41°45′03"	70°42′14"
0404	Downstream of 0401 in front of last house before Crab Cove Marina, sampled onshore	41°44′57"	70°42′04"
0405	50′ offshore of 0404	41°44′57"	70°42′06"
0406	100′ offshore of 0404	41°44′56"	70°42′09"
0407	In front of house just downstream of Warr's Marina, sampled onshore	41°45′09"	70°42′32"
0408	50′ offshore of 0407	41°45′09"	70°42′30"
0409	100′ offshore of 0407	41°45′09"	70°42′28"
0410	In front of house just before Broad Marsh Cove, sampled onshore	41°44′57"	70°42′33"
0411	50′ offshore of 0410	41°44′55"	70°42′32"
0412	100′ offshore of 0410	41°44′54"	70°42′29"

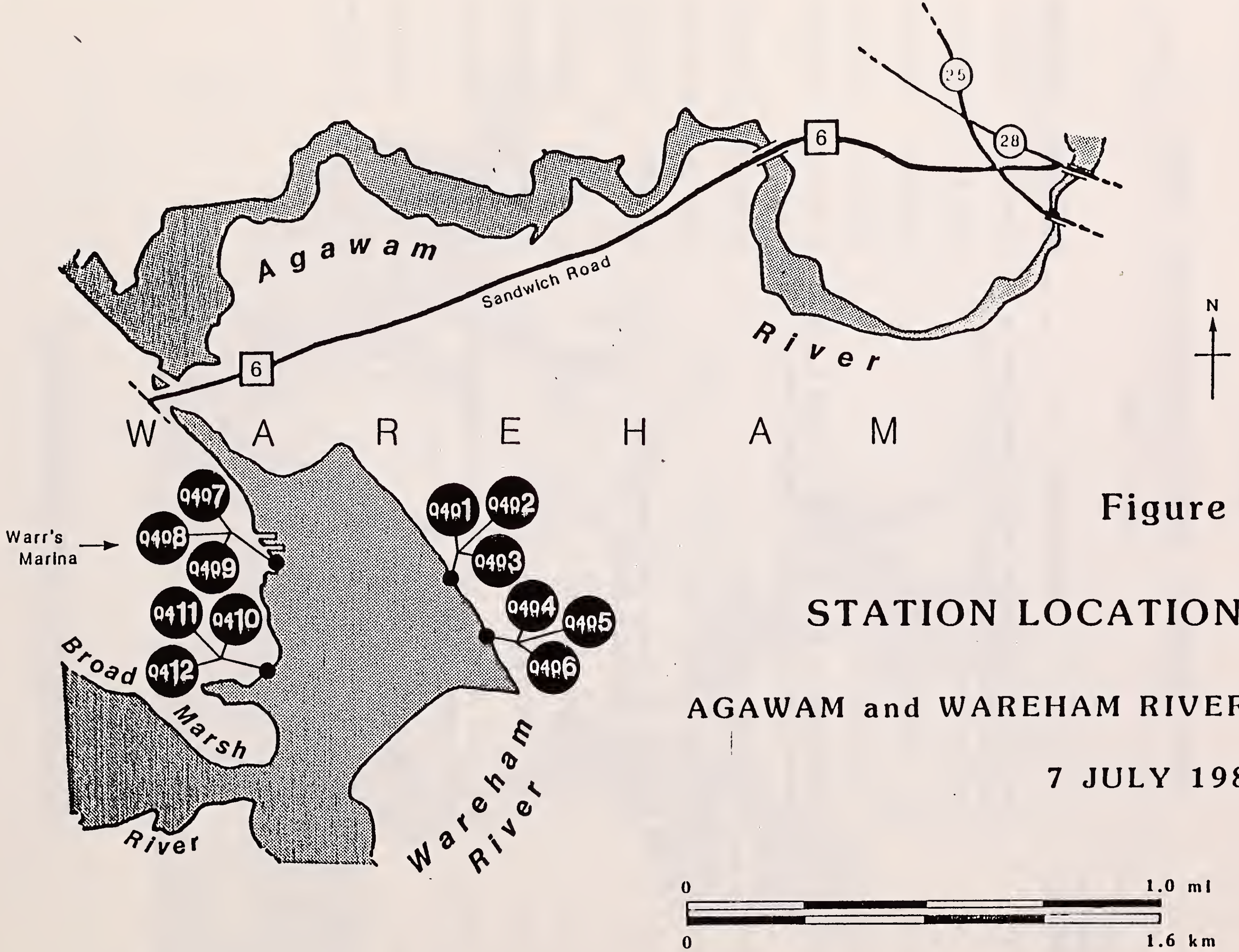

Figure A - 5

STATION LOCATIONS

AGAWAM and WAREHAM RIVERS

7 JULY 1986

TABLE A-12

STATION LOCATIONS - WAREHAM RIVER, WAREHAM

JULY 9, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
0501	Below railroad bridge on east side of Wareham River opposite British Landing Condominiums	41°41′16"	70°42′36"
0502	Approximately 50′ downstream of 0501	41°41′13"	70°42′35"
0503	Small cove near Oak Hill Road	41°45′19"	70°42′25"
0504	Near intersection of Oak Hill Road and Oak Street	41°45′18"	70°42′19"
0505	Above Crab Cove along Oak Street	41°44′55"	70°42′03"
0506	Just east and upstream of buoy 26	41°44′57"	70°42′08"
0507	Just downstream of Crab Cove close to Parkwood Drive	41°44′54"	70°42′13"
0508	Just downstream of buoy 26	41°44′54"	70°42′18"
0509	Just downstream of and west of buoy 26	41°44′50"	70°42′22"
0510	Just upstream of buoy 26	41°44′53"	70°42′29"

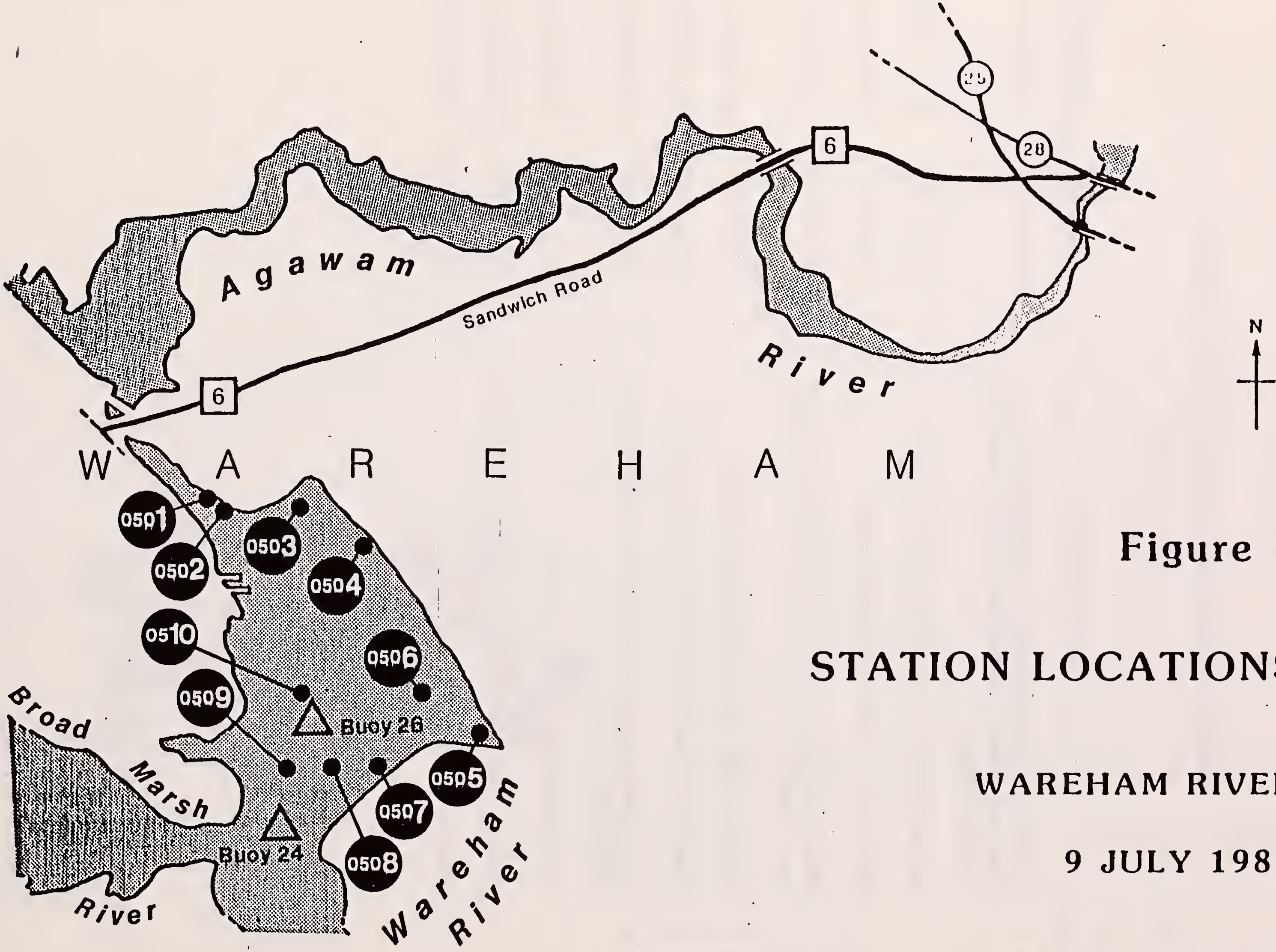

Figure A-6

STATION LOCATIONS

WAREHAM RIVER

9 JULY 1986

TABLE A-13

STATION LOCATIONS - EAST BRANCH OF THE WESTPORT RIVER, WESTPORT

JULY 14, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
0601	Snell Creek sampled at Jessie's Neck Cove	41°34'40"	71°00'35"
0602	Unnamed brook that enters Jessie's Neck Cove	41°34'45"	71°04'37"
0603	Along shore of farm at Pelegs Point	41°35'30"	71°04'15"
0604	Drainage ditch approximately 100' upstream from 0603	41°35'35"	71°04'13"
0605	Approximately 100' offshore of 0604	41°35'35"	71°04'10"
0606	Approximately 100' offshore of 0603	41°35'30"	71°04'13"
0607	Head of Kirby Brook Cove	41°35'45"	71°04'15"
0608	Head of Everett Cove	41°36'30"	71°03'45"
0609	Mouth of Everett Cove	41°36'35"	71°03'50"
0610	Just downstream of Hix Bridge, collected midstream	41°34'15"	71°04'30"

Figure A-7

STATION LOCATIONS

EAST BRANCH of WESTPORT RIVER

14 JULY 1986

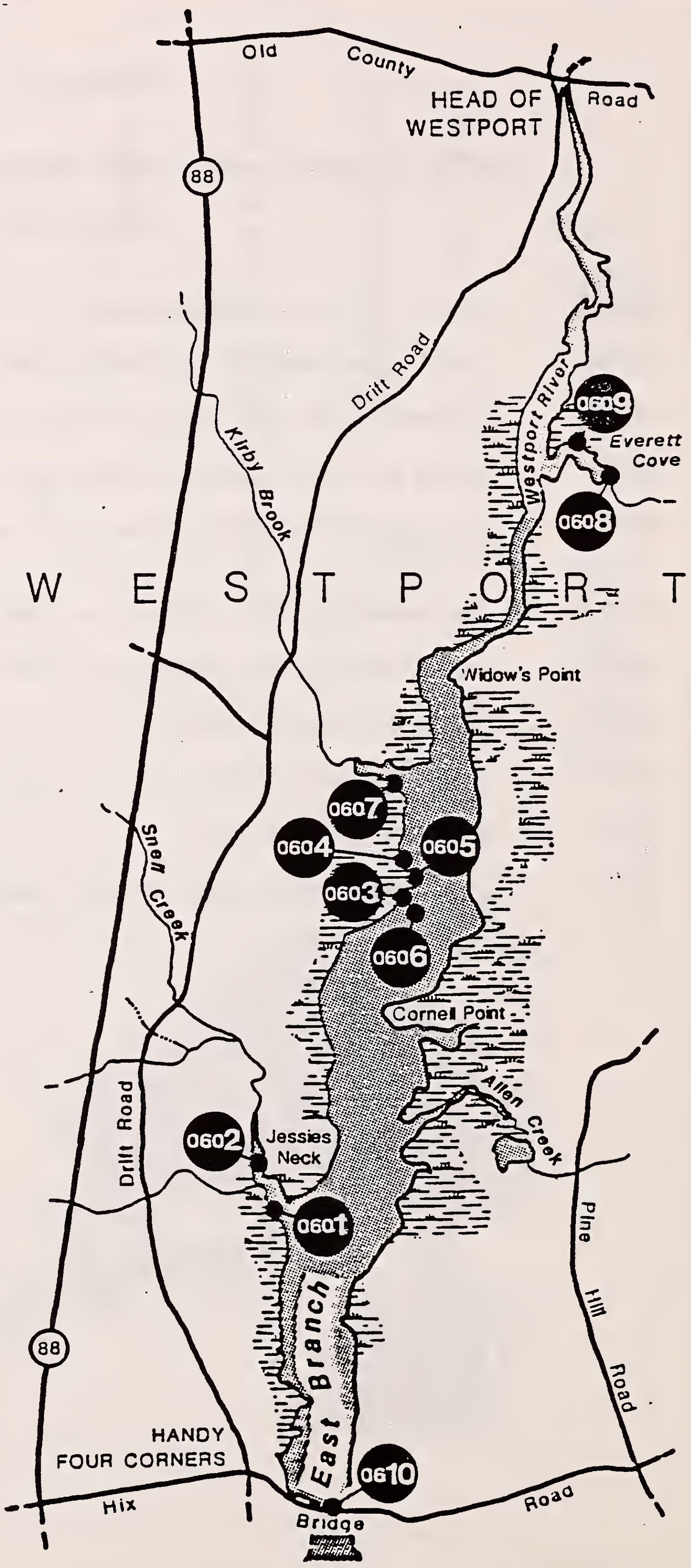

TABLE A-14

STATION LOCATIONS - WAREHAM RIVER, WAREHAM

JULY 21, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
0701	Off dock, below railroad bridge	41°45’25"	70°42’46"
0702	Opposite shore from 0701, approximately 20’ from shore	41°45’22"	70°42’43"
0703	Approximately 200’ downstream of 0702, just upstream of Cape Cod Shipbuilding	41°45’19"	70°42’39"
0704	Just upstream of British Landing Condominiums	41°45’13"	70°42’37"
0705	In front of British Landing Condominiums approximately 200’ from shore	41°45’12"	70°42’35"
0706	Opposite shore from 0705 in front of red garage, approximately 300’ from shore	41°45’21"	70°42’32"
0707	In front of Warr’s Marina, approximately 100’ off of the next to last slipway	41°45’10"	70°42’33"
0708	Just off of last slipway at Warr’s Marina	41°45’05"	70°42’33"
0709	Below Marina, in front of gray house, approximately 100’ from shore	41°45’04"	70°42’31"
0710	Opposite Marina, just past channel marker #22	41°44’51"	70°42’22"
0711	Onshore, just upstream of town landing	41°44’54"	70°42’06"
0712	Onshore, just downstream of town landing	41°44’53"	70°42’08"

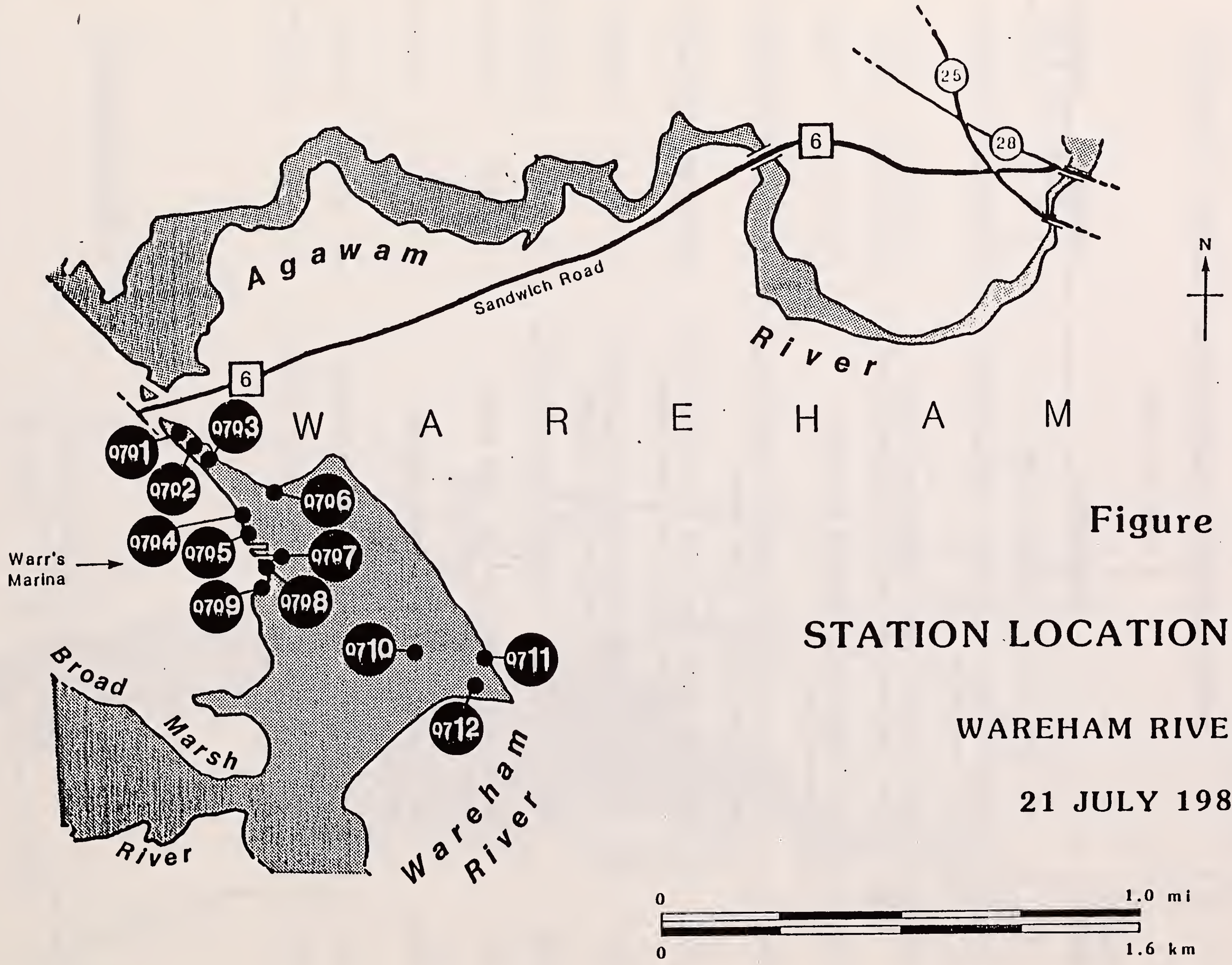

Figure A - 8

STATION LOCATIONS

WAREHAM RIVER

21 JULY 1986

TABLE A-15

STATION LOCATION - EAST BRANCH OF WESTPORT RIVER, WESTPORT

July 28, 1986

STATION NUMBER	DESCRIPTION	LATTITUDE	LONGITUDE
0801	Just downstream of Jessie's Neck, approximately 30' from western shore	41°34'51"	71°04'30"
0802	Same as 0801, approximately 150' from shore	41°34'50"	71°04'29"
0803	Mid-channel, opposite Jessie's Neck	41°34'49"	71°04'28"
0804	One-third of way off eastern shore, opposite Jessie's Neck	41°34'48"	71°04'27"
0805	Approximately 200' from eastern shore	41°34'47"	71°04'26"
0806	Approximately 40' from eastern shore	41°34'45"	71°04'25"
0807	Mid-channel just south Hix Bridge	41°34'15"	71°04'30"
0808	Just south Lakes Island	41°33'33"	71°04'18"
0809	Mouth Codman Cove, close to western shore	41°33'36"	71°03'39"
0810	Off of Little Pine Island in main channel	41°33'00"	71°03'13"
0811	Level with northern end of Great Island approximately 200' off of mainland	41°32'39"	71°03'09"
0812	Off northern end of Great Island	41°32'43"	71°03'40"

Figure A-9

STATION LOCATIONS

EAST BRANCH of WESTPORT RIVER

28 JULY 1986

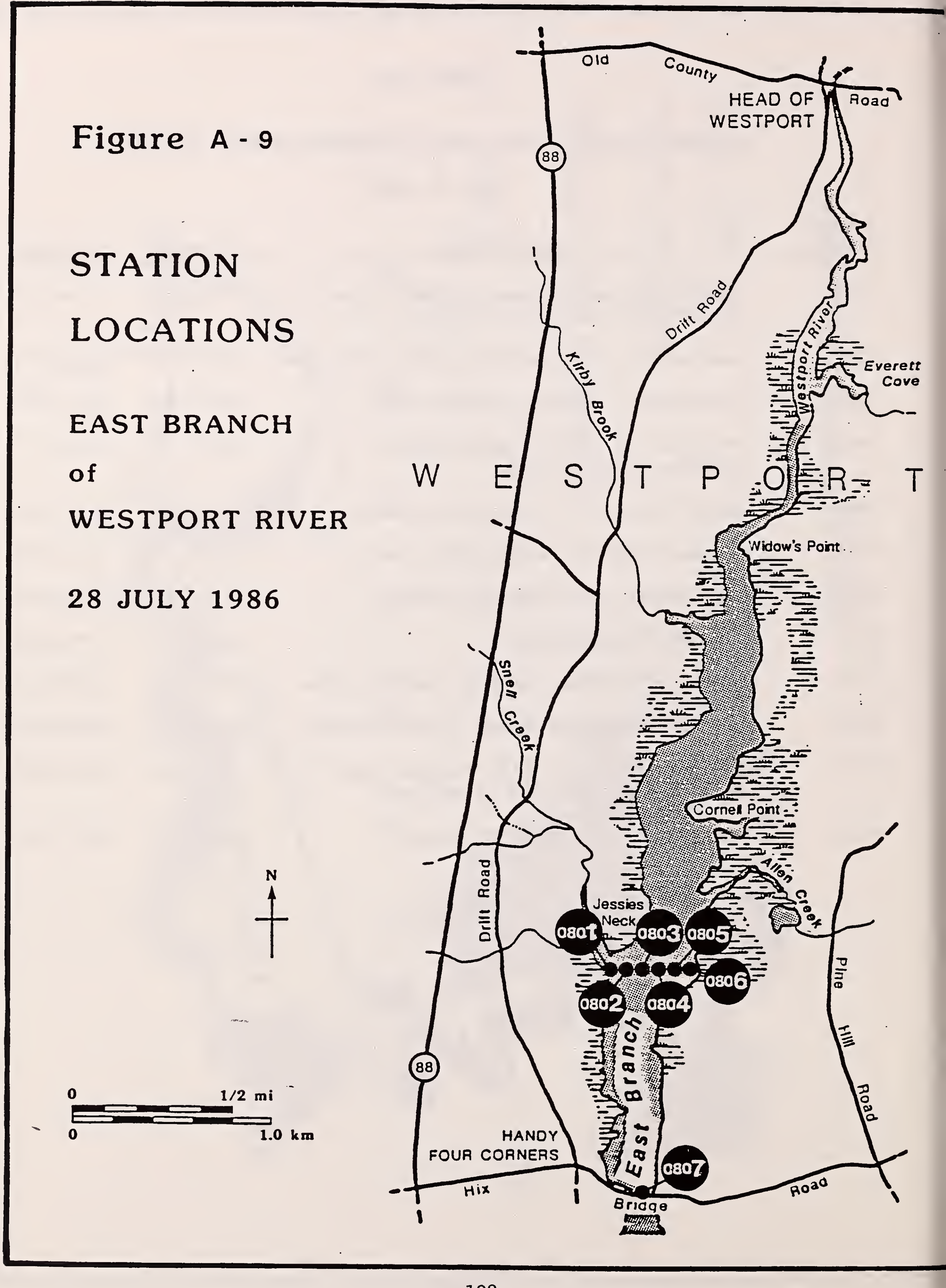

Figure A - 10 STATION LOCATIONS

EAST BRANCH of WESTPORT RIVER

(South of Hix Bridge)

28 JULY 1986

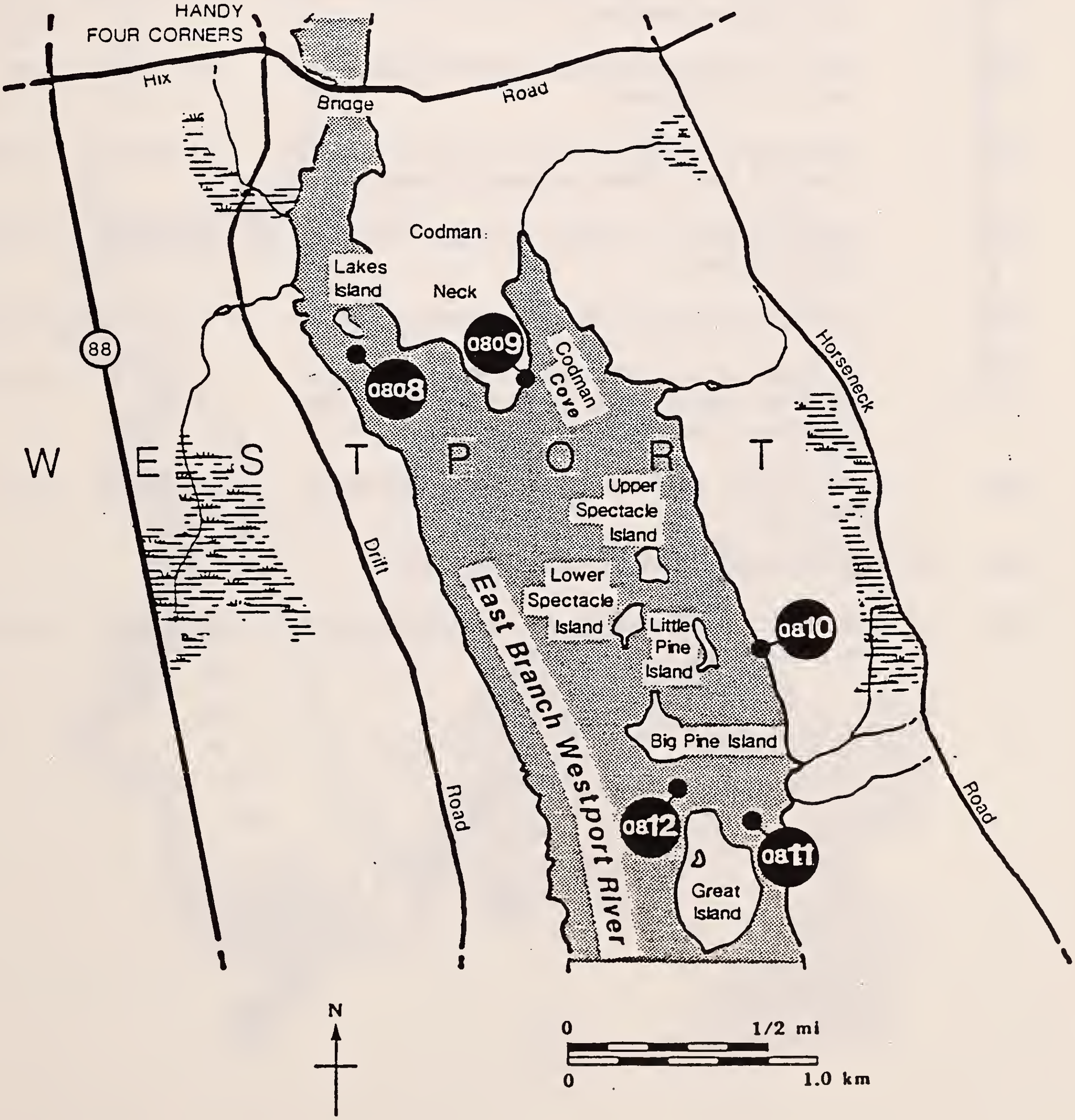

TABLE A-16

STATION LOCATIONS - WAREHAM RIVER, WAREHAM

AUGUST 3, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
0901	Just north of British Landing Condominiums	41°45'19"	70°42'41"
0902	In front of British Landing Condominiums, off slumping sea wall	41°45'18"	70°42'39"
0903	End of British Landing Condominiums, just north of the tower	41°45'15"	70°42'37"
0904	Storm drain flowing out of sea wall in front of boat crane at Warr's Marina	41°45'14"	70°42'37"
0905	First slipway in Warr's Marina, behind a cabin cruiser	41°45'11"	70°42'32"
0906	In next to last slipway at Warr's Marina	41°45'06"	70°42'31"
0907	In next to last slipway at Warr's Marina, Wareham, behind same boat as in 0905 two to three minutes later	41°45'06"	70°42'29"
0908	Just below last slipway behind a fishing boat	41°45'07"	70°42'31"
0909	Open water south of Warr's Marina		
0910	Chemical sample collected at same location as 0907	41°45'06"	70°42'29"

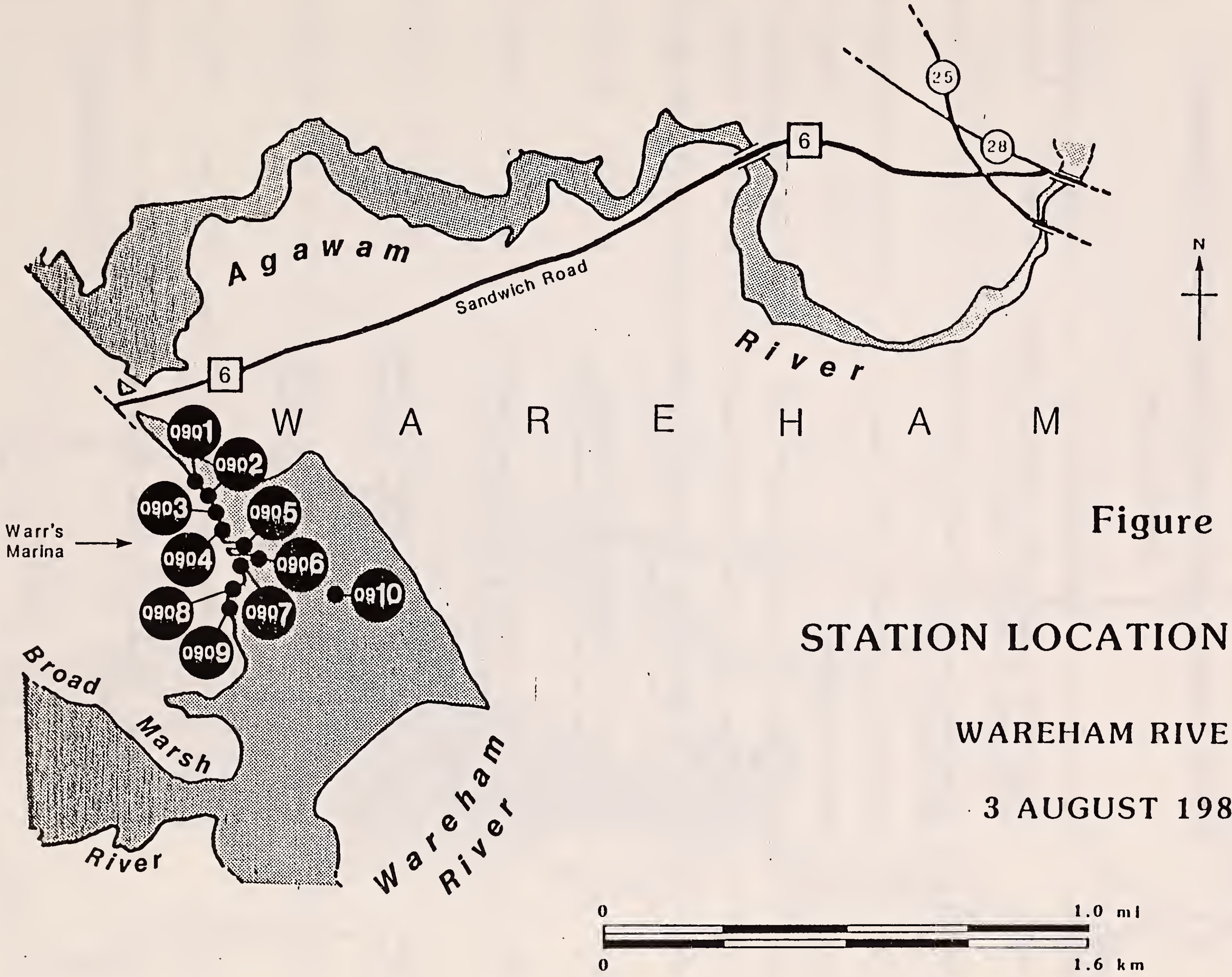

Figure A - 11

STATION LOCATIONS

WAREHAM RIVER

3 AUGUST 1986

TABLE A-17

STATION LOCATIONS - EAST BRANCH OF WESTPORT RIVER, WESTPORT

AUGUST 4, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
1001	North of Hix Bridge, just south of Widow Point, cove on western shore	41°35′50"	71°04′00"
1002	Opposite shore, approximately 100′ from shoreline	41°35′50"	71°04′03"
1003	Mid-channel just below Allen Creek	41°35′00"	71°04′15"
1004	Snell Creek Cove	41°34′40"	71°04′30"
1005	Mid-channel level with Jessie's Neck	41°34′35"	71°04′20"
1006	East side of river level with Jessie's Neck	41°34′30"	71°04′17"
1007	South of Hix Bridge, in channel by Upper Spectacle Island opposite cornfield	41°33′18"	71°03′26"
1008	In channel at southern end of Upper Spectacle Island, opposite house with flagpole	41°33′11"	71°03′22"
1009	Even with northern end of Lower Spectacle Island approximately 50′ from shore opposite cottages	41°33′07"	71°03′20"
1010	Even with middle Lower Spectacle Island approximately 50′ from shore, in front of yellow house	41°33′08"	71°03′17"
1011	Southern end of Lower Spectacle Island approximately 50′ from shore just south red cabin	41°33′02"	71°03′14"
1012	Just south of houses, opposite wooded area, approximately 50′ from shore	41°32′56"	71°03′11"
1013	In channel between Big Pine Island and Great Island	41°32′42"	71°03′25"

Figure A - 12

STATION LOCATIONS

EAST BRANCH of WESTPORT RIVER

4 AUGUST 1986

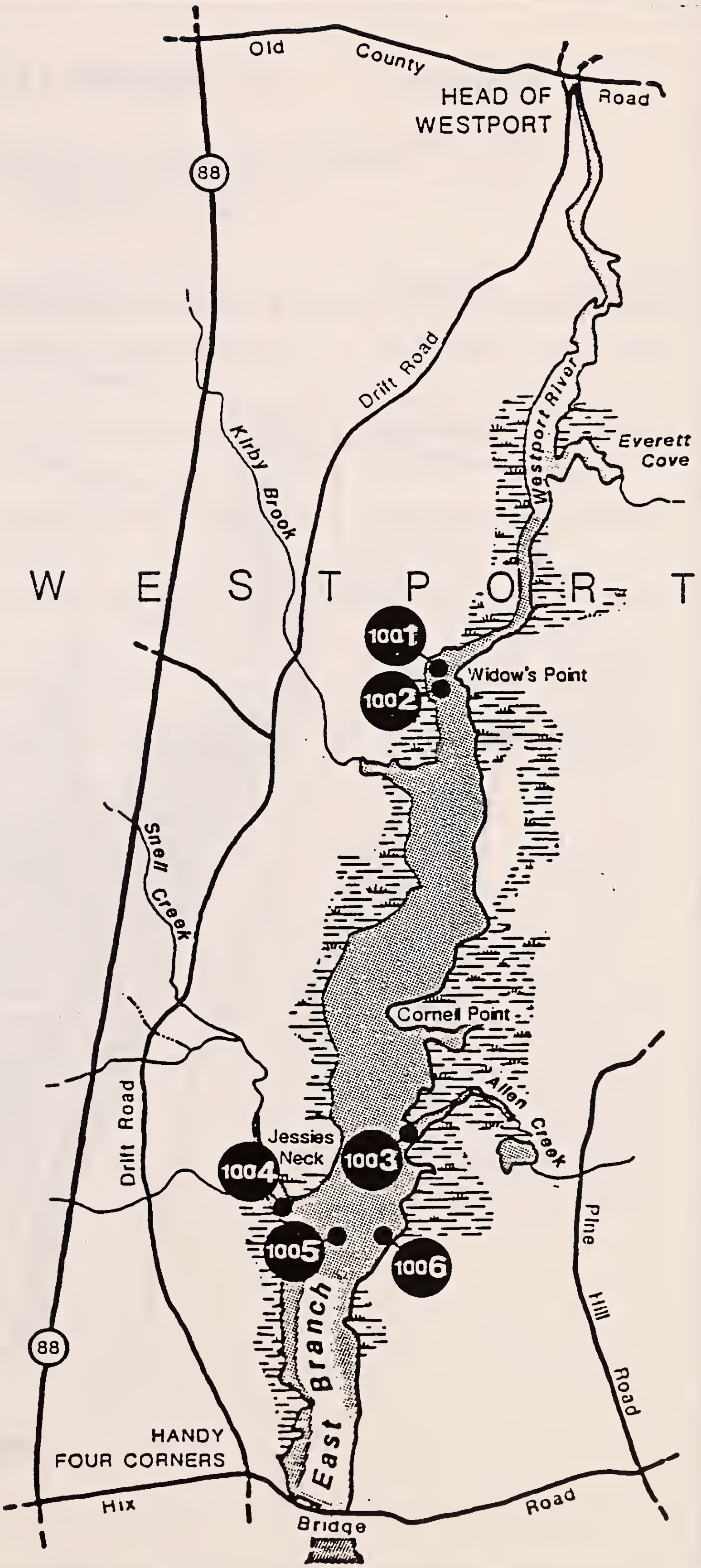

Figure A - 13

STATION LOCATIONS

EAST BRANCH of WESTPORT RIVER

(South of Hix Bridge)

4 AUGUST 1986

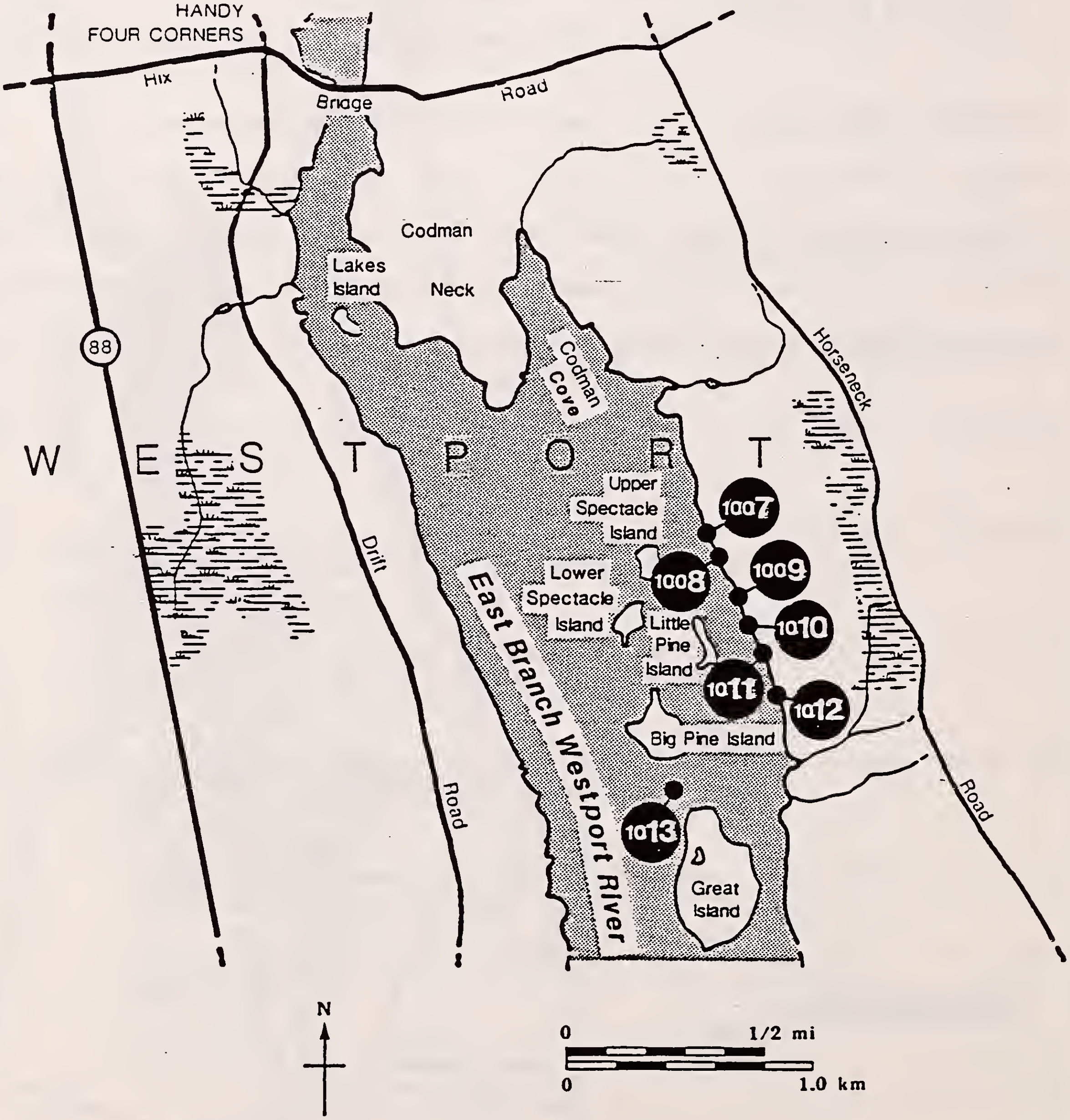

TABLE A-18

STATION LOCATIONS - ONSET BAY, WAREHAM

AUGUST 10, 1986

STATION NUMBER	DESCRIPTION	LATTITUDE	LONGITUDE
1101	West of Point Independence Yacht Club, approximately 200′ from shore	41°44′24"	70°38′16"
1102 to 1105	Between slipways at Point Independence Yacht Club	41°44′30"	70°38′16"
1106	Between Point Independence Yacht Club and Onset Bay Marina	41°44′34"	70°38′13"
1107 to 1113	Between slipways at Onset Bay Marina	41°44′38"	70°38′09"
1114	East of Onset Bay Marina	41°44′37"	70°37′58"
1115	Approximately 100′ off Monument Heights	41°44′34"	70°37′37"

Figure A - 14 STATION LOCATIONS

ONSET BAY 10 AUGUST 1986

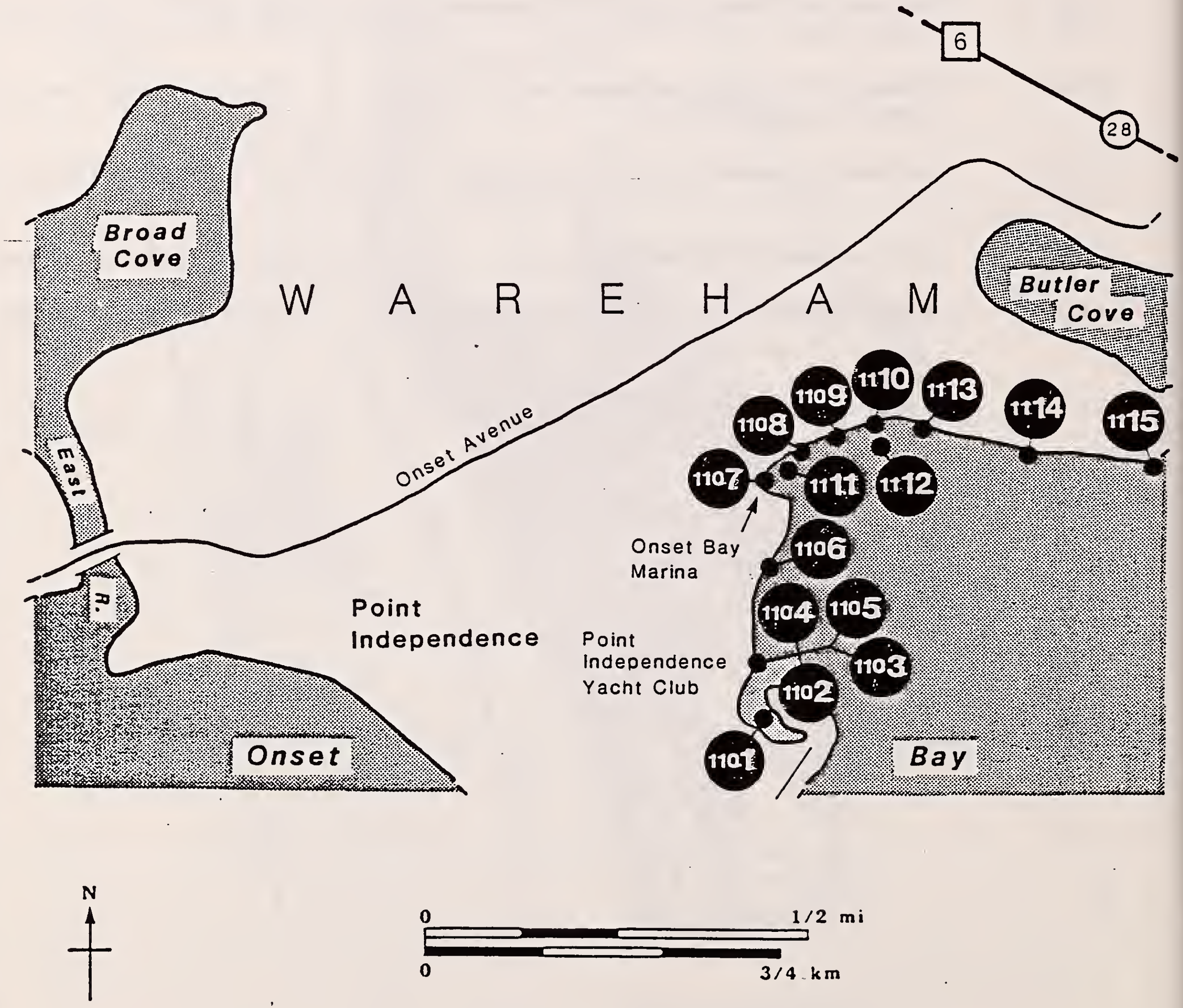

TABLE A-19

STATION LOCATIONS - MUDDY COVE, WAREHAM

AUGUST 11, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
1201	Unnamed brook on western shore of Muddy Cove	41°44′55"	70°39′38"
1202	Mid-way up unnamed brook	41°44′56"	70°39′41"
1203	Wind row in unnamed brook	41°44′55"	70°39′44"
1204	Unnamed brook which drains Beaverdam Pond	41°45′12"	70°39′38"
1205	Gibbs Brook by Ocean Spray factory, approximately 100′ from culvert	41°45′22"	70°39′06"
1206	Same as 1205, approximately 25′ from culvert	41°45′19"	70°39′07"
1207	Small cove by Ocean Spray factory	41°45′18"	70°39′06"
1208	Confluence of unnamed brook with the cove on eastern shore of Muddy Cove	41°45′15"	70°39′06"
1209	Approximately 100′ upstream of 1208	41°45′14"	70°39′04"
1210	Just south of last bend in channel in Muddy Cove	41°44′56"	70°39′33"
1211	Just south of Dummy Bridge, collected midstream	41°44′50"	70°39′09"
1212	Gibbs Brook behind Cranberry Greens Mini-Golf	41°45′25"	70°39′16"

Figure A - 15

STATION LOCATIONS

MUDDY COVE

11 AUGUST 1986

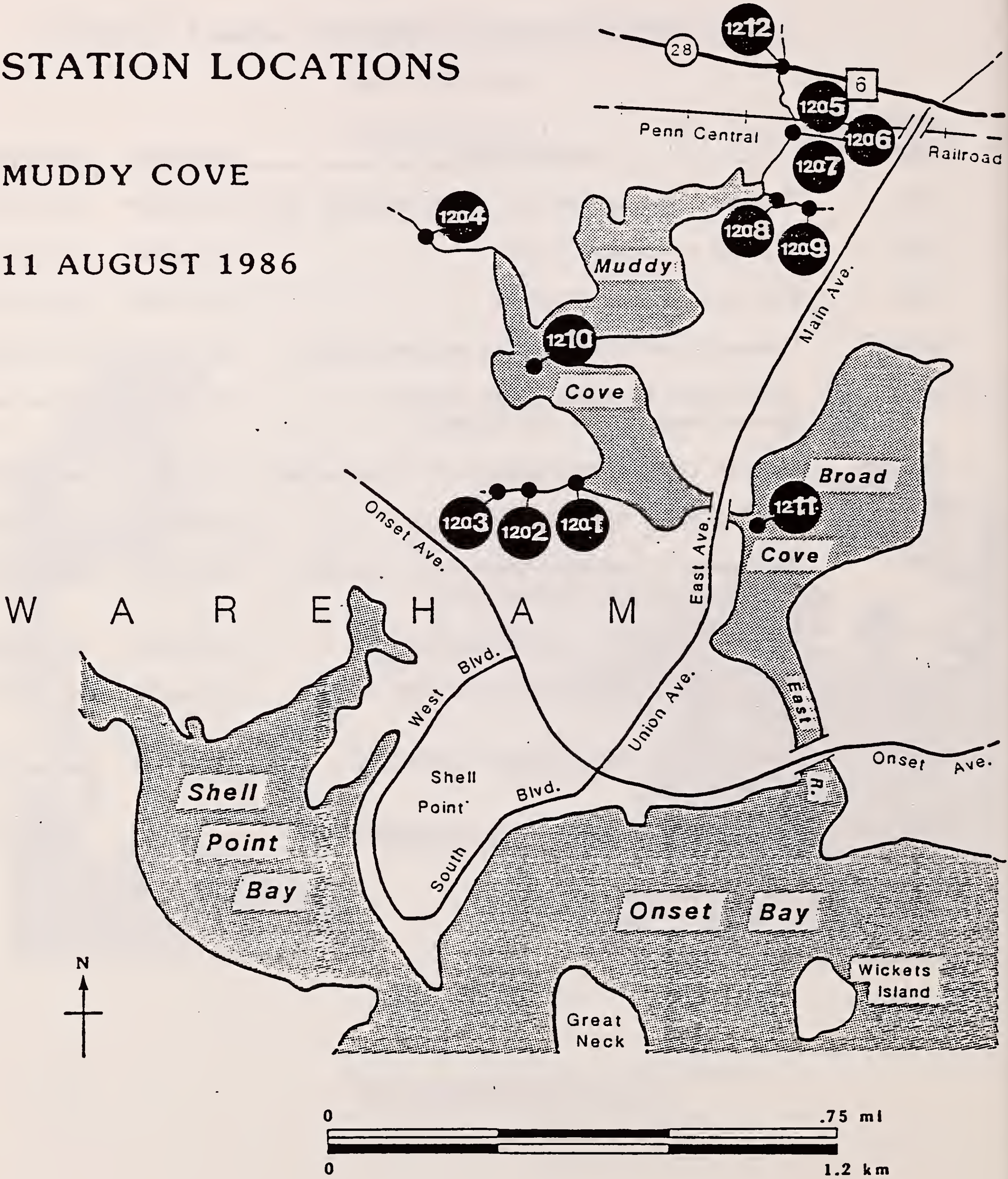

TABLE A-20

STATION LOCATIONS - EAST BRANCH OF WESTPORT RIVER, SOUTH OF HIX BRIDGE, WESTPORT

AUGUST 19, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
1301	Below Hix Bridge along western shore, next to wetland, approximately 20′ from shore, above housing area	41°33′52"	71°04′28"
1302	Unnamed tributary, just north of Gull Rock	41°33′48"	71°04′29"
1303	Below first two houses, just north of first pier	41°33′40"	71°04′26"
1304	In front of house with gabled roof, approximately 20′ from shore just south of first pier	41°33′38"	71°04′26"
1305	In front of second house with flagpole, white house with beach wall	41°33′36"	71°04′23"
1306	Just south of first group of houses, opposite wooded area below rocky shoals	41°33′29"	71°04′18"
1307	On eastern shore opposite meadow, approximately 50′ from shore	41°33′14"	71°03′21"
1308	Just south of red house with TV dish, approximately 50′ from shore	41°33′08"	71°03′15"
1309	Opposite white cottage, two other small cottages north of it	41°33′06"	71°03′13"
1310	Opposite white two story house	41°33′04"	71°03′12"
1311	Just south of gray cottage	41°33′02"	71°03′11"
1312	Just south of last house, opposite southern end of Little Pond Island	41°32′58"	71°03′10"

Figure A - 16

STATION LOCATIONS

EAST BRANCH of WESTPORT RIVER

(South of Hix Bridge)

19 AUGUST 1986

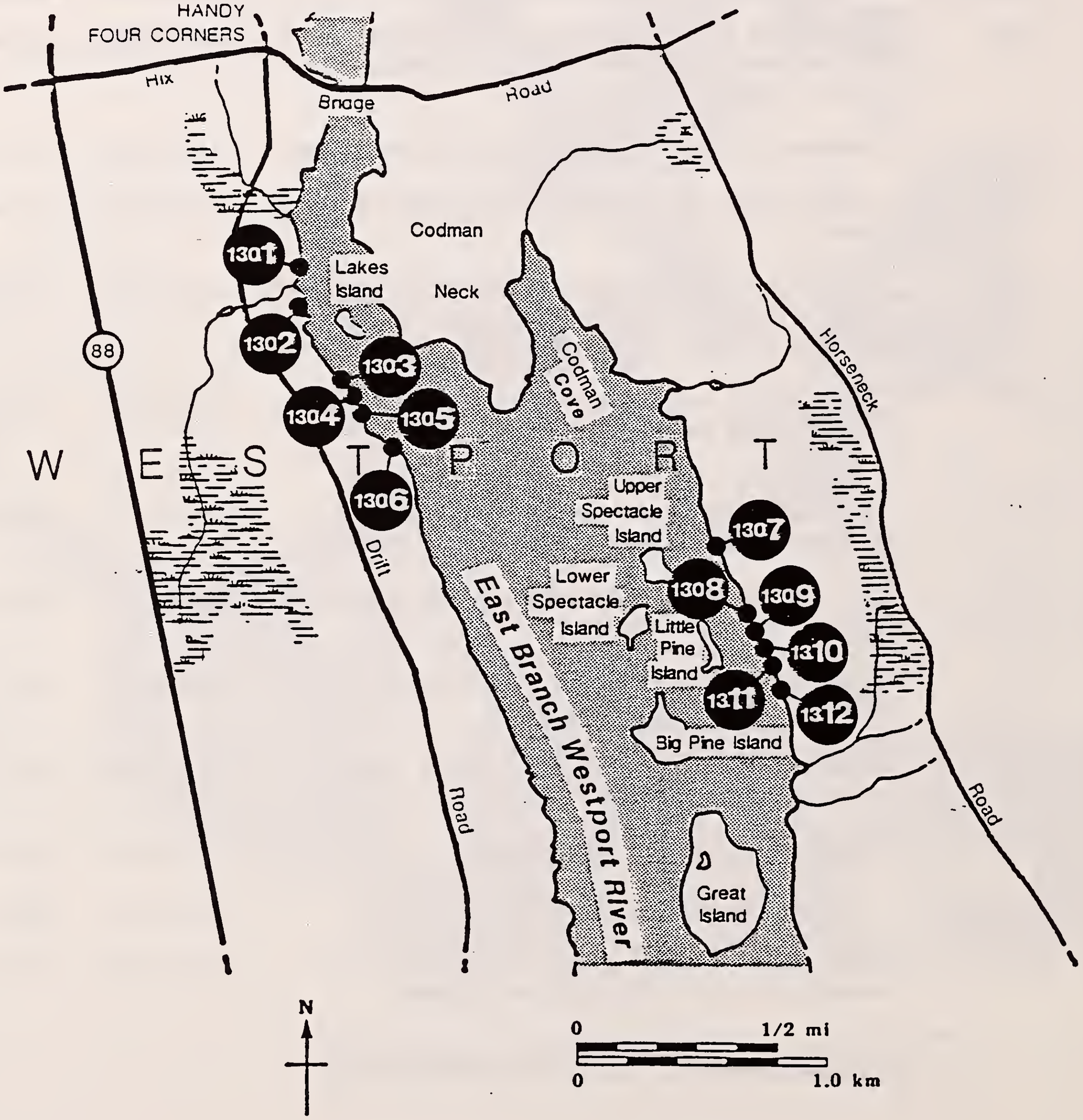

TABLE A-21

STATION LOCATIONS - SUNSET COVE, WAREHAM

AUGUST 26, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
1401	Up major unnamed tributary in Broad Marsh, approximately 1/8 of a mile from where it crosses Great Neck Road	41°44′56"	70°39′39"
1402	Approximately half way down same tributary to Broad Marsh in Sunset Cove	41°45′04"	70°39′41"
1403	Mouth of tributary, approximately 100′ from shore	41°45′05"	70°39′44"
1404	Southern shore of cove near Helminth Avenue	41°45′17"	70°39′49"
1405	Small cove near Northport Road and Central Avenue, on southern side of Broad Marsh Cove	41°45′27"	70°39′16"
1406	Sunset Cove by Onset Avenue	41°44′42"	70°39′56"
1407	Northern tip of Sunset Island, in front of group of seven houses close to shore	41°44′39"	70°40′00"
1408	Offshore of Sunset Island	41°45′16"	70°39′17"
1409	Backside of Sunset Island near West Avenue and Second Street	41°45′17"	70°39′15"

Figure A - 17 STATION LOCATIONS

SUNSET COVE 26 AUGUST 1986

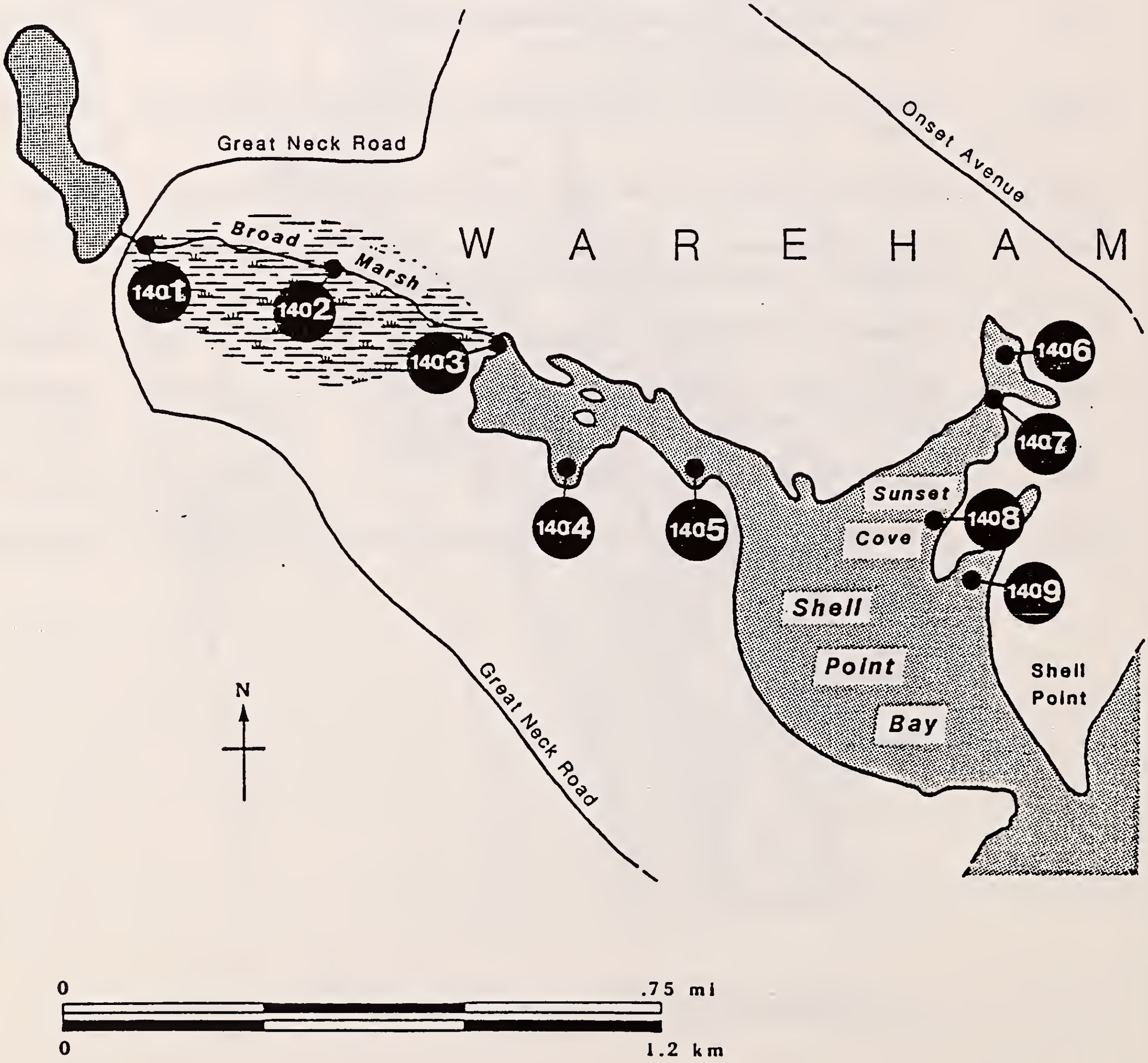

TABLE A-22

STATION LOCATIONS - EAST BRANCH OF WESTPORT RIVER, WESTPORT

SEPTEMBER 17, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
1501	Head of Jessie’s Neck Cove	41°35’05"	71°04’30"
1502	Mouth of Jessie’s Neck Cove	41°34’45"	71°04’27"
1503	Offshore of Cornell Point	41°35’15"	71°04’20"
1504	Drainage ditch at Cornell Point	41°35’17"	71°04’18"
1505	Drainage ditch southwest of Peleg’s Point	41°35’20"	71°04’10"
1507	Outlet of Kirby Brook	41°35’40"	71°04’12"
1508	Mouth of Everett Cove	41°36’15"	71°03’45"
1509	Cove opposite Peleg’s Point	41°35’20"	71°03’50’
1510	Unnamed stream north of Allen Creek	41°35’15"	71°04’10"

Figure A - 18

STATION LOCATIONS

EAST BRANCH
of
WESTPORT RIVER

17 SEPTEMBER 1986

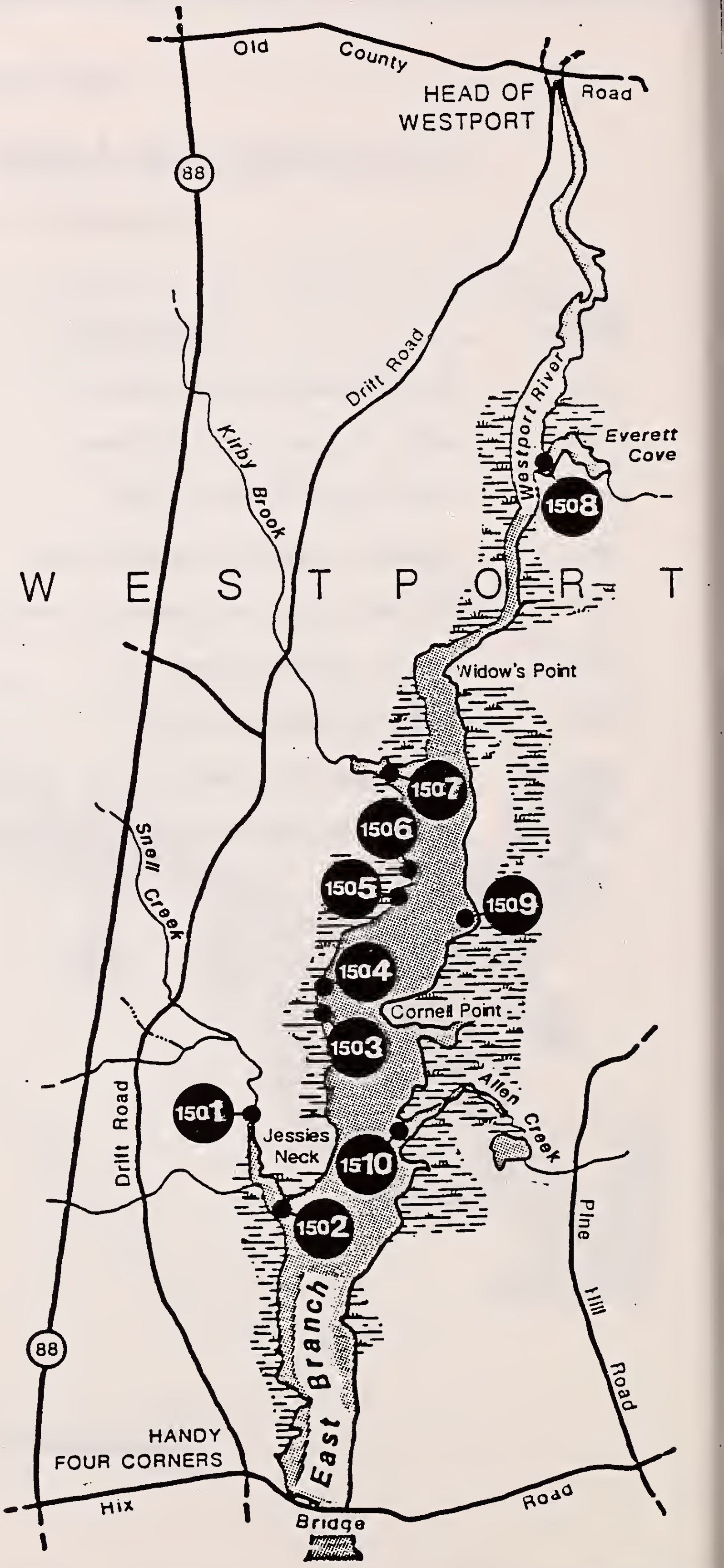

TABLE A-23

STATION LOCATIONS - SUNSET COVE, WAREHAM

SEPTEMBER 24, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
1601	Unnamed stream approximately one-half way between Broad Marsh Cove and Great Neck Road	41°44′51"	70°40′57"
1602	Unnamed stream near confluence with Broad Marsh Cove	41°44′46"	70°40′46"
1603	Small cove near Verve Avenue sampled approximately 20′ from shore	41°44′39"	70°40′47"
1604	Cove near Northport Avenue	41°44′34"	70°40′28"
1605	Southeastern section of cove by Onset Avenue in northeast corner of Sunset Cove	41°44′39"	70°39′55"
1606	Same cove as in 1605, northwestern section approximately 200-300′ to Western Avenue and houses	41°44′43"	70°39′58"
1607	In front of houses northern tip of Sunset Island closest to Onset Avenue, approximately 15′ from shore	41°44′38"	70°40′00"
1608	In front of houses southwestern end of Sunset Island, approximately 15′ from shore	41°44′30"	70°40′08"
1609	Inner side of Sunset Island near 5th Street	41°44′32"	70°39′57"
1610	Inner side of Sunset Island near 2nd Street	41°44′27"	70°40′03"

Figure
A - 19

STATION LOCATIONS

SUNSET COVE 24 SEPTEMBER 1986

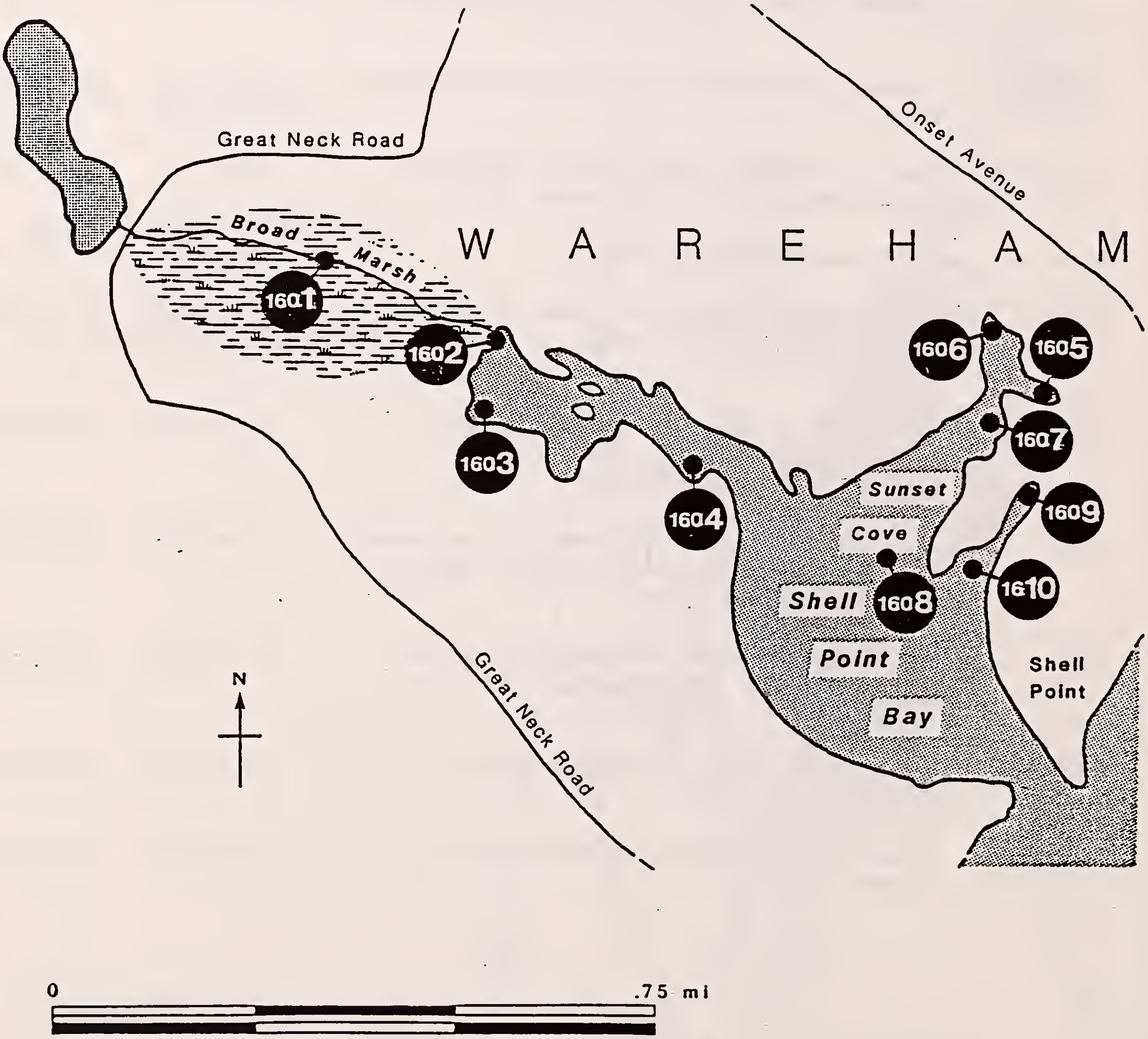

TABLE A-24

STATION LOCATIONS - EAST BRANCH OF WESTPORT RIVER, WESTPORT

OCTOBER 2, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
1701	Unnamed tributary at Drift Road just north Snell Corner	41°35'10"	71°05'00"
1702	Opposite dead end road off of Drift Road just north of Snell Corner at unnamed tributary	41°35'15"	71°04'58"
1703	Head of Jessie's Neck Cove, sampled from dirt road	41°35'10"	71°04'35"
1704	Approximately 300 feet upstream of 1703, sampled from bridge	41°35'10"	71°04'36"
1705	Entrance to Jessie's Neck Cove	41°34'45"	71°04'35"
1706	Jessie's Neck Cove just south of 1703	41°35'02"	71°04'35"
1707	Confluence Snell Creek with mainstem Westport River at Jessie's Neck Cove	41°34'40"	71°04'33"
1708	Eastern shore of Westport River opposite Peleg's Point	41°35'30"	71°04'00"
1709	Cove just south of 1708	41°35'29"	71°04'00"
1710	Unnamed tributary to Snell Creek just above its confluence with Snell Creek, same tributary as 1702	41°35'30"	71°04'40"
1711	Snell Creek just above confluence with unnamed tributary sampled in 1710	41°35'14"	71°04'40"
1712	Drift Road at Snell Creek	41°35'20"	71°04'45"

Figure A - 20

STATION LOCATIONS

EAST BRANCH
of
WESTPORT RIVER

2 OCTOBER 1986

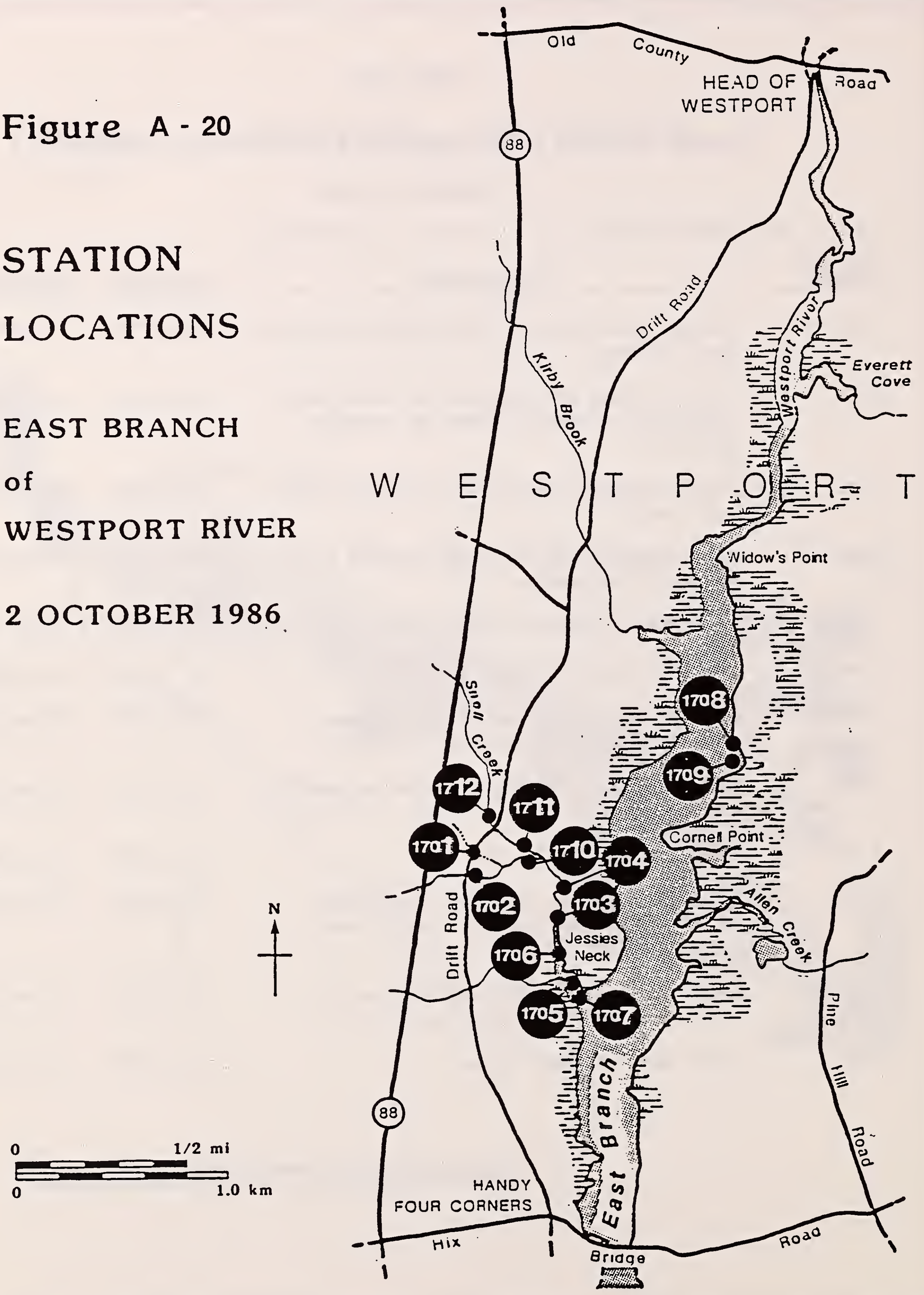

TABLE A-25

STATION LOCATIONS - BROAD COVE AND EAST RIVER, WAREHAM

OCTOBER 8, 1986

STATION NUMBER	DESCRIPTION	LATITUDE	LONGITUDE
1801	Broad Cove just north of Dummy Bridge	41°44′53"	70°39′19"
1802	Broad Cove at outfall on northwestern shore along Riverside Drive	41°44′58"	70°39′13"
1803	Approximately 60′ to right of 1802	41°45′02"	70°39′10"
1804	Head of Broad Cove	41°45′04"	70°39′07"
1805	Along south eastern shore of Broad Cove, in front of house near Pleasantville Rd., approximately 20′ from shore	41°44′58"	70°39′05"
1806	At second outfall in little cove near Sandlewood Lane, approximately 50′ from shore	41°44′54"	70°39′05"
1807	At East River, near Sandlewood Lane, approximately 50′ from shore	41°44′41"	70°39′18"
1808	Center of cove at town boat ramp on western shore of Broad Cove	41°44′40"	70°39′20"

Figure A-21

STATION LOCATIONS

BROAD COVE

and EAST RIVER

8 OCTOBER 1986